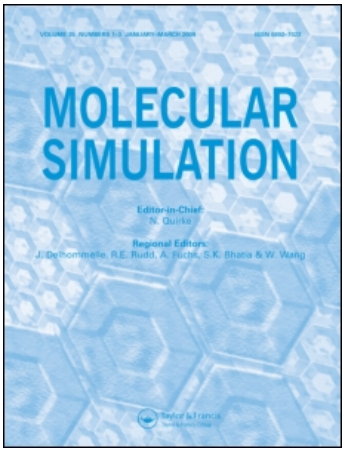

Molecular Simulation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

A Modified Valley Restrained Monte Carlo Method to Efficiently Search the Low Energy Structures of Peptides

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To cite this Article Chai, Chong Chul , Choi, Seung Hoon and Jhon, Mu Shik(2000) 'A Modified Valley Restrained Monte Carlo Method to Efficiently Search the Low Energy Structures of Peptides', Molecular Simulation, 25: 6, 385 — 398
To link to this Article: DOI: 10.1080/08927020008028168
URL: http://dx.doi.org/10.1080/08927020008028168

Molecular Simulation, 2000, Vol. 25, pp. 385–398
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A MODIFIED VALLEY RESTRAINED MONTE CARLO METHOD TO EFFICIENTLY SEARCH THE LOW ENERGY STRUCTURES OF PEPTIDES

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(Received December 1999; accepted December 1999)

A new Monte Carlo sampling scheme, namely the Modified Valley Restrained Monte Carlo procedure, is used to obtain the global energy minimum conformations for polypeptides, such as Met-enkephalin and Melittin. For each peptide, we found close agreement with previous results from both theoretical and experimental studies. The simple idea for controlling the step size according to the Valley Function, provides useful suggestions in searching the global energy minimum structures, and furthermore helps solve the multiple minima problem.

Keywords: Simulation; Monte Carlo; Valley Function; variable step size; global optimization

1. INTRODUCTION

A full understanding of protein functions requires knowledge of their three-dimensional structure. Unfortunately, experimentally determined structures for most proteins are unavailable. Consequently, it is essential to develop approaches for predicting secondary and tertiary structures of proteins.

In the late 1950s Anfinsen and his colleagues made a remarkable discovery [1]. They were exploring a long-standing puzzle in biology. What causes

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newly made proteins to wind into specifically shaped forms able to perform crucial tasks in the living cell? In the process of discovery the team found the answer was simpler than anyone had imagined. It was the sequence of amino acids, referred to as the primary structure of the protein, that determines the native conformation. This structure is stable under physiological conditions and has a biological activity.

This successful experiment of refolding the ribonuclease by Anfinsen *et al.* [1], has evoked a new fundamental subject in predicting the tertiary structure of proteins theoretically from the sequence of its amino acids. It is now assumed that the native conformation of a protein is that which corresponds to the global minimum for a potential energy function. This global optimization of protein and polypeptide structure has its own formidable problems due to the high complexity of their energy hyper-surface; *i.e.*, multiple minima problem. The typical number of local minima in the potential energy surface varies as 10^n, where n is the number of residues of polypeptides. Thus a direct searching of the global energy minimum structure in the whole conformational space, quickly becomes impossible as the number of residues increases.

In order to get over this multiple minima problem, many theoretical works for the computing the stable conformations of proteins have been developed [2–8]. These previous works can be classified into two categories; an improvement in the methodology of computation, and the simplification of the interaction potential function. But yet there is none which can be regarded as a close solution to the problem. There still should be much research to find it.

Since the Monte Carlo method simulates the thermal Markovian processes, it might be applicable to this problem of obtaining a global energy minimum structure for a protein. However a straightforward application of the conventional Metropolis method for a protein system has proven to be very inefficient [9–11]. Many previous researchers have tried to improve the efficiency of the conventional Metropolis Monte Carlo Method, and to also overcome the energy barrier between local minima [12–20].

One of the main difficulties is the isotropic sampling of the Metropolis Monte Carlo procedure without considering the anisotropic potential energy surface of proteins. If we properly take into account this anisotropic energy surface in the progression of the simulation steps, we may say hopefully that the Monte Carlo method will become a powerful method to simulate the globular structural changes of proteins.

Valley Restrained Monte Carlo (VRMC) [15], that predicts the topology of the potential surface using both statistical and empirical data, has an

additional Preselection Step. This step carries out a preferential sampling of the region near the energy minima and the valley between minima. However, in spite of the efficient sampling ability of VRMC, the Preselection Step (*a priori* selection step) induces a little bias, because of a defect where sampling of phase space cannot cover all the conformational space in the main MC step.

The main idea of this work is that (1) knowledge about the statistical properties of amino acids is used in an optimal way, (2) the moving boundary (step size) of the next set is controlled by the Valley Function of the previous set, (3) although the search still continuously covers all the conformational space, the search for the statistically important regions of a conformational space is reinforced more than the search for other conventional procedures.

We do control MC moving boundary, according to the Preselection Surface obtained the Valley Function. The Valley Function which passes through the local minima and then goes along the valley between local minima on the hyper-surface, is formulated from the acquired Protein Data Bank (PDB) in advance. VF is the most probable pathway for crossing the barrier in the process of simulation, because it is the curve which connects two minima by way of the lowest energy barrier. And then, we can suppose the step size as a function of the Preselection Surface obtained from VF. The higher the Preselection Factor, which is the value obtained from the Preselection Surface for the arbitrary (ϕ, ψ) zone, then the higher the probability that the system also exists near the local energy minimum. Then, it can cross over the energy barrier more efficiently, by increasing the step size near the valley region. We will explain about the analytical form of both the Preselection Surface as well as the Valley Function in the next section.

The method is tested with the well-studied pentapeptide, Met-enkephalin, and 20 residue segment of Melittin, using CVFF (Consistent Valence Force Field) potential parameters [22] in order to describe its pair-wise atomic interactions. To estimate the efficiency of the method, it is further compared with a "classical" optimization method: Metropolis Monte Carlo procedure (MMC).

2. METHODOLOGY

The Ramachandran maps of 20 amino acids from the selected 101 protein data sets were obtained from Brookhaven Protein Data Bank. The selected

proteins have a resolution equal to or higher than 3.0 Å. The average length of sequences is 226. Though there are some differences in the number of data for each amino acid according to its natural abundance, the average number of data for one amino acid is 1136. This is sufficient to manipulate statistically.

The following is a brief summary to get the Valley Function (VF). The Valley Function (VF) can be derived from a back-bone torsion set of selected protein structures as shown in previous paper [15]. Equation (1) describes the Valley Function which optimally passes through torsional zones (ϕ, ψ) of individual residues.

$$\begin{aligned} \mathrm{VF}(\phi, \psi) = 1 &+ b_1\phi + b_2\psi + b_3\phi^2 + b_4\phi\psi + b_5\psi^2 \\ &+ b_6\phi^3 + b_7\phi^2\psi + b_8\phi\psi^2 + b_9\psi^3 \end{aligned} \tag{1}$$

The coefficients $(b_1, \ldots, b_9)$ can be determined in Eq. (1) by using least square methods (Eqs. (2,3)). The coefficients have been listed as shown in the original paper [15].

$$\mathrm{Error}(b_1, \ldots, b_9) = \sum_{i=1}^{n} \{\mathrm{VF}(\phi_i, \psi_j)\}^2 \tag{2}$$

$$\left[\frac{\partial\{\mathrm{Error}(b_1, \ldots, b_9)\}}{\partial b_j}\right]_{j=1,\ldots,9} = 0 \tag{3}$$

The value moves zero for a point on the VF curve, while the absolute value increases as the point far from the curve. For an arbitrary torsional set (ϕ, ψ), we can then define both the "Preselection Surface" and "Preselection Factor", as determined by Eq. (4). If the chosen torsional set (ϕ_0, ψ_0) is on the VF curve, the Preselection Factor ($\mathrm{PS}(\phi_0, \psi_0)$) is therefore one, because $\mathrm{VF}(\phi_0, \psi_0)$ is equal to zero whereas the Preselection Factor decreases and finally converges to zero, as the torsional set deviates from the VF. The equation form is the same with the "Preselection Surface" of VRMC, except the steepness factor (η) exists outside of the exponential function.

$$\mathrm{PS}(\phi, \psi) = \eta \exp\{-\mathrm{VF}^2\} \tag{4}$$

where PS is ranged from 0 to η. η is the steepness factor which controls the smoothness of the Preselection Surface.

In the "Near" Valley Region, where individual residues of protein structures exist with higher probability, it is thought that an unknown target system may have many local minima and with high energy barriers. Then, it is reasonable to sample torsional phase space with a relatively large step size. On the contrary, supposing that a "Far away" Valley Region has a more steep Potential Energy Surface with relatively lower energy barriers, step size in this region can also be controlled in order to decrease it according to the Preselection Factor.

As a result, we can search lower energy structures with more sampling efficiency. This is shown the schematic picture in Figure 1.

We average out the Preselection Factor for back-bone torsion in a previous MC set, then we can resize the moving boundary for the next (k) set in the product form of the initial step size (δ_0) and Preselection factor. This average out for each torsion in the previous ($k-1$) set, as shown in Eq. (5). The Preselection Surfaces of individual residues are obtained from Eq. (4).

$$\delta^k = \frac{\delta_0 \eta}{M} \sum_{m=1}^{M} [\exp\{-\mathrm{VF}_m(\phi_i, \psi_i)^2\}]^{k-1} \tag{5}$$

where $\mathrm{VF}_m(\phi_i, \psi_i)$ is the Valley Function for a back-bone dihedral angle (ϕ_i, ψ_i) of m-th set and η is the steepness factor.

We will describe the implementation in detail.

1. The total steps M_{tot} are divided by M, the number of steps per set, to give M_{tot}/M sets. M should be chosen so that the information on the different local energy minima do not mix together and therefore lose statistical

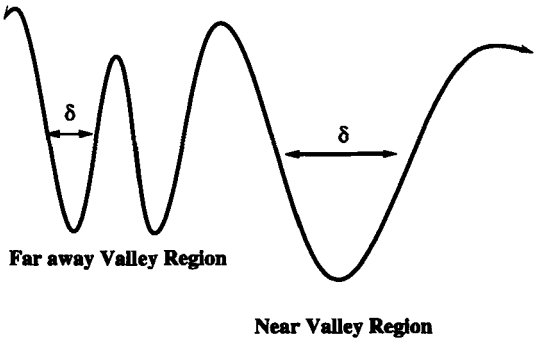


FIGURE 1 A schematic picture of variable step size δ, dependent on Valley Region.

significance. M values can be obtained by monitoring the convergence of conformational fluctuations, and it is found that $M = 20 \sim 30$ MCS (Monte Carlo Steps per particle or torsion) is adequate.

2. Prepare the initial structures of different conformations and perform the minimization for $1000 \sim 1500$ step using an appropriate potential function in order to avoid the non-bonded crash.
3. Temperature annealing from 1000 K to the room temperature using 3% annealing rates. This temperature range and the annealing rate were justified by analyzing the vibrational normal mode frequencies by Shin and Jhon [10]. The temperature of the $(k-1)$ th set, which is composed of a number of Monte Carlo steps, is given by

$$T^k = T^{k-1} - \chi_T T^{k-1} \tag{6}$$

where χ_T is the temperature annealing factor.

4. Determine the step size of k-th set using the averaged Preselection Factor in the $(k-1)$-th set.

$$\delta_i^k = \delta_0 \cdot \langle \mathrm{PS}(\phi_i, \Psi_i) \rangle_M^{k-1} \tag{7}$$

where index (i) indicates the individual torsion number.

5. Generate new configuration $(\phi_{\mathrm{new}}, \psi_{\mathrm{new}})^k$ in the k-th set.

$$\theta_{\mathrm{new}}^k = \theta_{\mathrm{old}}^k + \delta_i^k \cdot R \quad R \in [1, -1] \tag{8}$$

6. Conventional Metropolis test is performed through computing the energy difference $(\Delta E = E(\phi_{\mathrm{new}}, \psi_{\mathrm{new}}) - E(\phi_{\mathrm{old}}, \psi_{\mathrm{old}}))$.

$$\begin{aligned} &\Delta E \leq \text{accept the new configuration} \\ &\Delta E > \text{reject or accept by Boltzmann Factor } \exp(-\Delta/kT) \end{aligned} \tag{9}$$

7. Return to Step 3 with the new configuration and iterate the process.

When the Monte Carlo method is applied to find the minimum energy structure of a molecule, the geometry of the molecule is then usually treated as rigid; *i.e.*, bond lengths and bond angles are fixed and only the torsional degrees of freedom are taken into account.

3. RESULTS AND DISCUSSION

To compare the Modified Valley Restrained Monte Carlo (MVRMC) with the conventional Metropolis Monte Carlo (MMC), we carried out simulations for two peptides, Met-enkephalin and Melittin.

3.1. Met-enkephalin

Met-enkephalin is a pentapeptide hormone in the mammalian brain with morphine-like activities [23].

$$H-Tyr^1-Gly^2-Gly^3-Phe^4-Met^5-OH$$

The initial structure was prepared in the full extended form, minimized for 1000 steps using the steepest descents method. We carried out the several runs to find the optimal value for the steepness factor (η) of a Preselection Surface at 300 K, by taking an average conformational change per step $\langle \Sigma_i (\Delta\theta_i)^2 \rangle^{1/2}$ as an indicator of the sampling efficiency. This is conceptually very similar to the rms differences between the current structure and the next structure. Through the preliminary simulation runs for 400 K steps, the optimal steepness factor was determined by ~ 1.8. And initial step size was set to 20 degrees in order to get the optimal acceptance ratio ($0.45 \sim 0.55$). After the steepness factor was determined, we then performed the main simulation for a 20000 set with the factor (η). Because one set was composed of 20 MCS (Monte Carlo steps per a torsion), the overall procedure was composed of 800 K steps.

Figure 2 shows the energy equilibration process in the simulation. This figure clearly shows the increased power of MVRMC as compared to that of MMC. In the case of MMC, the system may be trapped in local minima during the full simulation, while MVRMC makes it possible to escape from the local minima. In the energy profile of MVRMC, there is a large energy change at ~ 3000 step. This fact indicates that while MMC could not escape from the local minima within current MC steps, MVRMC however enables the system to overcome the energy barrier and avoid being trapped in the local minima. The acceptance ratios are 0.4578 and 0.5225 for the MMC and MVRMC, respectively. It is remarkable that the acceptance ratio of MVRMC is larger than MMC, though the averaged step size slightly increase by ~ 3.4 degrees than MMC. It comes from the fact that the sampling of MVRMC is more efficient searching the lower energy surfaces.

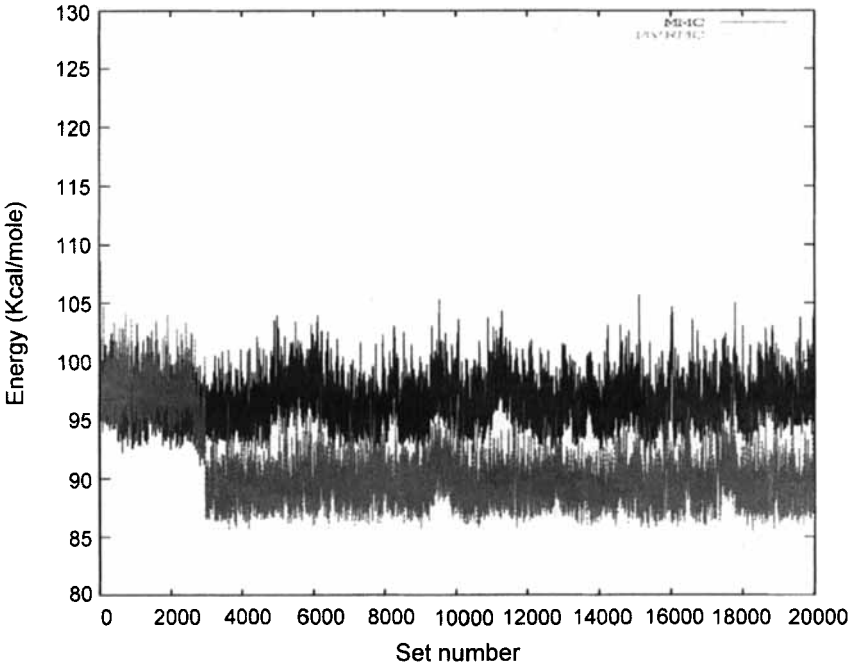


FIGURE 2 A comparison of the energy equilibration process(kcal/mole) between MMC and MVRMC for Met-enkephalin, where it is reported per every set, and one set is composed of 400 steps.

TABLE I A comparison of principal torsion angles for Met-enkephalin

	Torsional angles (in degree)		
Residue	ϕ	ψ	ω
Tyr^1	−59.03(−83.50)*	122.13(155.80)	−169.31(−177.20)
Gly^2	−129.88(−154.31)	87.04(86.00)	168.19(168.50)
Gly^3	86.21(82.92)	−76.30(−75.10)	−178.81(−169.98)
Phe^4	−141.13(−136.89)	19.08(19.12)	−178.66(−174.09)
Met^5	−132.30(−163.50)	160.08(161.20)	−175.66(−179.80)

*The values in parenthesis are the results of previous works [24].

In the Table I, the results of the MVRMC simulation are compared with that of MMC. The MVRMC structure is consistent with that of a previous report [24] which shows that the global energy minimum structure of Met-enkephalin is a II′β-bend type of structure. Another simulation from random starting point gives similar results, although it is not shown in this report.

3.2. Melittin

Melittin is the principal component of the venom from honey bees, with 26 amino acid residues and six positive and no negative charges. The conformation of the dimer of the melittin from X-ray data [27–29] is shown in Figure 3. NMR experimental results indicate that the six C-terminal residues do not influence the conformation of the 20 N-terminal [25, 26]. We simulate the 20-residue fragment of melittin with the sequence

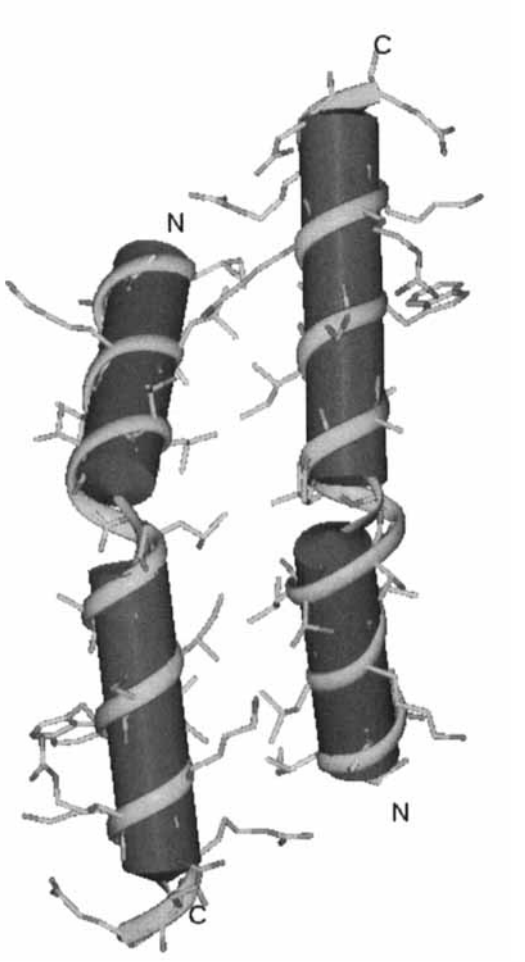


FIGURE 3 The crystal structure of Melittin dimer found by X-ray crystal diffraction.

as follows;

GIGAVLKVLTTGLPALISWI

where each of the characters represent the one-letter abbreviation of an amino acid.

Clearly, the structure is composed of two helices jointed by a "hinge-like" structure between the Tyr^{11} and Gly^{12} residues. The side chains are distributed such that the polar side chains point to the convex part of the molecule, while the non-polar side chains point to the concave part. And melittin is known to be either unordered or having just a trace of helix in water without salt at a neutral pH and at low concentrations. However in high protein concentrations and/or in a solution with high ionic strength, it adopts a largely helical conformation and aggregates as a tetramer. We applied the MVRMC method to locate the lowest energy conformation for monomeric melittin in a deprotonated form, which mimics the conformation at higher pH.

At first, we select the starting structure such that all the torsion angles of the main chain are $(-90, 0)$, which is the saddle point between two secondary structures, α-helix and β-sheet. From the view point of reducing the convergence time, taking a starting conformation at a saddle point is very effective to reduce the negative effects of the initial condition.

TABLE II A comparison of principal torsion angles of the lowest energy minimum conformation for Melittin 2–19 segments

Residue	*Torsional angles (in degree)*	
	ϕ	ψ
Ile^{2}	−61.78(−65.86)*	−40.48(−28.65)
Gly^{3}	−45.31(−70.27)	−45.30(−60.00)
Ala^{4}	−65.49(−56.74)	−46.39(−44.43)
Val^{5}	−46.05(−62.28)	−63.37(−44.42)
Leu^{6}	−55.76(−63.11)	−47.18(−37.03)
Lys^{7}	−56.24(−62.60)	−52.32(−43.83)
Val^{8}	−58.31(−61.00)	−39.15(−49.73)
Leu^{9}	−80.69(−53.87)	−30.57(−35.29)
Thr^{10}	−83.99(−73.50)	−29.60(−19.17)
Thr^{11}	−110.68(−75.13)	−40.74(106.30)
Gly^{12}	−47.66(108.52)	−61.83(−78.05)
Leu^{13}	−61.35(−67.87)	−55.20(−65.12)
Pro^{14}	−53.17(−60.19)	−48.12(−35.76)
Ala^{15}	−62.26(−58.49)	−46.93(−43.70)
Leu^{16}	−56.13(−60.47)	−52.79(−53.97)
Ile^{17}	−56.36(−63.41)	−47.64(−27.17)
Ser^{18}	−55.23(−68.24)	−37.52(−47.68)
Trp^{19}	−67.11(−66.34)	−35.83(−50.62)

*The values in parenthesis are the results by the simulation of X-ray.

The steepness factor is determined to 1.4 through preliminary simulation, which is slightly smaller value than in the case of Met-enkephalin because of the complexity of Potential Energy Surface. The total simulations were performed for 1840 K steps, *i.e.*, 1000 set, with one set consisting of 1840 steps. Other conditions are analogous to those of Met-enkephalin.

The molecule easily converges to the minimum energy structure (full α-helix structure), which is identical to a previous X-ray structure. The resultant principal torsion angles are shown in Table II, and then compared with the simulation of the X-ray structure and MMC. The structure of MMC cannot reach to a full α-helix form, as expected.

Figure 4 shows the energy profile of MMC and MVRMC during the simulation. This figure shows that MVRMC always searches lower energy surfaces than does MMC. The structure from the MMC is trapped in the local minimum structure, so as not to reach a full α-helix structure, whereas that from the MVRMC reaches to the full helix structure, as shown Figure 5.

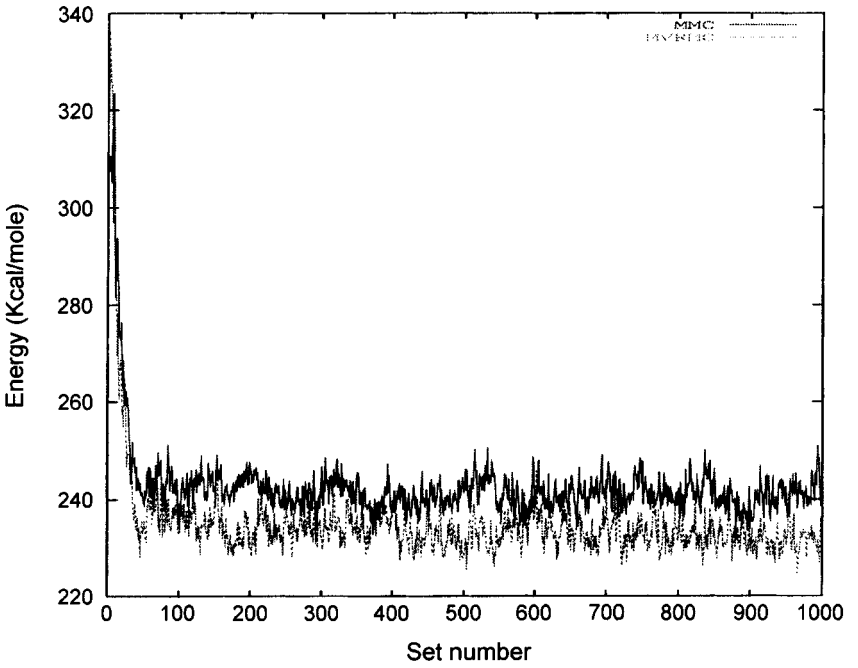
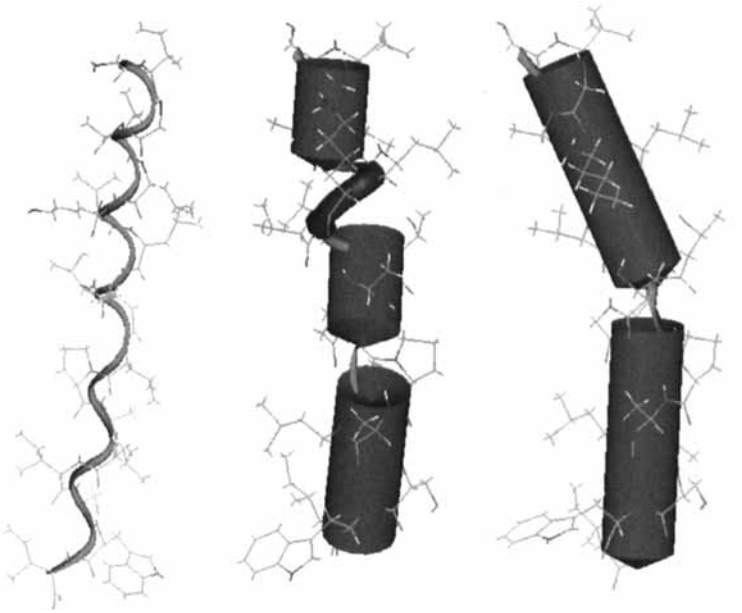


FIGURE 4 A comparison of the energy equilibration process (kcal/mole) between MMC and MVRMC for Melittin, where it is reported per every set, and one set is composed of 1840 steps.



(a) Starting Structure (b) MMC Simulation (c) MVRMC Simulation

FIGURE 5 A comparison of lowest energy structures from MVRMC and MMC.

4. CONCLUSION

In this paper, we present the Modified VRMC procedure, which is directly implemented into the Metropolis procedure by controlling the step size. Although the method is the same in the view of using the topology of the energy surface of the Valley Function, MVRMC though can remove the bias in sampling process. The MVRMC procedure was implemented successfully in the peptides system (Met-enkephalin and Melittin) in order to search a lower energy minimum structure.

Recently, 3-D structures of the numerous proteins have become known due to the advancement of X-ray or NMR experiment technology. Because this MVRMC is based on the known 3-dimensional structures of proteins, the accuracy of the Valley Function will be improved, and then the power of MVRMC will be known.

Acknowledgement

This work was supported in part by the Korea Research Center for Theoretical Physics and Chemistry.

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