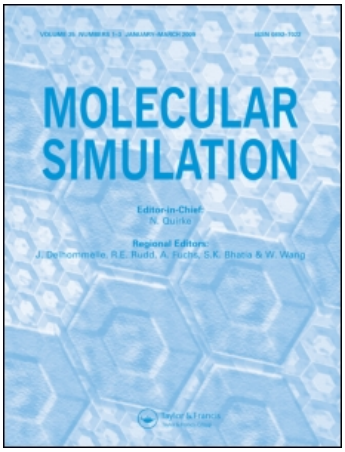

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Multiple Time Step Brownian Dynamics for Long Time Simulation of Biomolecules

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Multiple Time Step Brownian Dynamics for Long Time Simulation of Biomolecules

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We report a multiple time step algorithm applied to an atomistic Brownian dynamics simulation for simulating the long time scale dynamics of biomolecules. The algorithm was based on the original multiple time step method; a short time step was used to keep faster motions in local equilibrium. When applied to a 28-mer ββα folded peptide, the simulation gave stable trajectories and the computation time was reduced by a factor of 160 compared to a conventional molecular dynamics simulation using explicit water molecules. We applied it for the folding simulation of a 13-mer α-helical peptide, giving a successful folding simulation. These results indicate that the Brownian dynamics with the multiple time step algorithm is useful for studies of biomolecular motions by long time simulation.

Keywords: Brownian dynamics; Multiple time step; Molecular dynamics; Molecular simulation

INTRODUCTION

In order to understand living systems at molecular level, a development of computational framework that describes the dynamic processes in terms of physics and chemistry is essential. Molecular dynamics (MD) simulations have provided important insights into structural, dynamical and thermodynamic properties of biomolecules [1,2] since the first report of the MD simulation of a protein, bovine pancreatic trypsin inhibitor (BPTI) [3]. In MD simulation, the Newtonian equations of motions of atoms of macromolecules and the surrounding solvents are integrated numerically. The integration time step is approximately one order of magnitude smaller than the fastest motion. This generally limits the integration time step to the order of 1 fs. Now computers are increasingly fast, MD simulations of bigger systems are performed using more realistic systems and longer timescales [4–6]. Although nanosecond simulations are now routinely performed, they are still too short to observe many interesting biomolecular motions, such as protein folding and binding that occur on the microsecond to second timescale.

To overcome this limitation, many approaches have been proposed, which can be classified into two main categories: Using specialized computer and development of new algorithms. Examples of the former are the IBM Blue Gene Project [7], MDM (Molecular Dynamics Machine) [8], MD-Engine [9] and Folding@Home worldwide distributed computing project [10–14]. Constrained and reduced-variable simulation methods, namely SHAKE [15], RATTLE [16] and LINCS [17] algorithms, are typical examples of the latter approach. And multicanonical, simulated tempering and replica-exchange methods [19] are also used to sample much wider phase space.

Stochastic dynamics simulations, namely Langevin and Brownian dynamics [20,21], are another approach to overcome the conformational sampling problem. In those methods, water molecules are not treated explicitly and the influence of solvent particles on the solute is incorporated through additional frictional and random terms in a manner consistent with physical law. In Brownian dynamics (BD) simulations, since motions are over-damped, longer time steps are capable (more than MD and Langevin dynamics simulations). In fact, the time step of 10 fs could be used with LINCS algorithm and adaptive time step method (i.e. short time step is used whenever the energy of configurations is larger than predetermined threshold) in atomistic BD simulations reported by us [22] and

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Shen *et al.* [23]. However, much larger time step could not be applied for the BD simulation developed by us because of numerical instability.

In this report, we developed an algorithm for applying multiple time step idea to the Brownian equation of motion to achieve the more efficient conformational sampling.

The slower motions and random force acting as the heat bath are integrated with long time step, while the faster motions are integrated with the time step that keeps them in local equilibrium. In other words, BD is performed using "normal" long time step Δt, while this single step dynamics is composed of n iterations using short time step $\Delta\tau = \Delta t/n$ to retain faster motions in equilibrium. We test this algorithm applying to simulation of a 28-mer ββα folded peptide and folding of a 13-mer α-helical peptide. This is the first report of the folding simulation of an α-helical peptide using an atomistic Brownian dynamics algorithm.

THEORY

Brownian Dynamics Algorithm

By treating the effects of solvent as a dissipative random force, the Langevin equation can be expressed as

$$m_i \frac{\mathrm{d}^2\mathbf{r}_i}{\mathrm{d}t^2} = -\zeta_i \frac{\mathrm{d}\mathbf{r}_i}{\mathrm{d}t} + \mathbf{F}_i + \mathbf{R}_i. \tag{1}$$

Here, $\mathbf{r}_i$ and m_i represent the mass and position of atom i, respectively. $\mathbf{F}_i$ is the systematic force on atom i. $\mathbf{R}_i$ is a random force on atom i having a zero mean $\langle \mathbf{R}_i(t) \rangle = 0$ and a variance $\langle \mathbf{R}_i(t)\mathbf{R}_j(0) \rangle = 6\zeta_i k_\mathrm{B} T \delta_{ij}\delta(t)$, where k_B is the Boltzmann constant and T is the system temperature; this derives from the effects of solvent. ζ_i is a frictional coefficient and is determined by the Stokes' law:

$$\zeta_i = 6\pi a_i^{\mathrm{Stokes}} \eta = m_i \gamma_i, \tag{2}$$

where a_i^{Stokes} is a Stokes radius of atom i and η is the viscosity of water, γ_i is a collision frequency (or the damping constant) of atom i. γ^{-1} can be considered as the time taken for the particle to lose memory of its initial velocity (the velocity relaxation time).

In the region where the product of the integration time step Δt and $\gamma(\gamma\Delta t) \gg 1$, then if the systematic force is assumed to be constant over Δt, the following integration algorithm is obtained [20,21];

$$\mathbf{r}_i(t + \Delta t) = \mathbf{r}_i(t) + \frac{\mathbf{F}_i}{\zeta_i}\Delta t + \sqrt{\frac{2k_\mathrm{B}T}{\zeta_i}\Delta t}\boldsymbol{\omega}_i \tag{3}$$

where $\boldsymbol{\omega}_i$ is a random noise vector obtained from Gaussian distribution. Therefore, the integration time step is determined by the balance between the rate of change of the systematic force and γ, since the influence of the random force can be integrated over Δt.

Multiple Time Step Algorithm

Basic idea of multiple time step is introduced by Streett *et al.* in 1978 to reduce the computational cost of molecular simulations [18]. An alternative formulation of a multiple time step method is developed by Tuckerman *et al.* in 1992 as the "reversible reference system propagation algorithm (r-RESPA)" [24].

In this study, the forces were classified into two groups: Fast forces, $\mathrm{F_{fast}}$, and slow forces, $\mathrm{F_{slow}}$, with corresponding time steps, $\Delta\tau$ and Δt, respectively. The time steps are related via the ratio, $\Delta\tau = \Delta t/n$. We grouped the forces as follows:

$\mathrm{F_{fast}}$ = bonds, bond angles, dihedral angles, improper torsion angles, 1–4 van der Waals, and 1–4 electrostatic terms.
$\mathrm{F_{slow}}$ = 1–5 van der Waals, 1–5 electrostatic, hydrogen bonding and solvation terms.

Random force was treated as $\mathrm{F_{slow}}$ since the BD algorithm described above is applied under the assumptions that the systematic force to be constant during Δt and $\Delta t \gg \gamma^{-1}$.

In the simulation using the multiple time step algorithm, each step of the dynamics involves the following iterations:

1. $\mathrm{F_{slow}}$ is evaluated. Random force term is calculated using the time step of Δt.
2. $\mathrm{F_{fast}}$ is evaluated.
3. Propagation of the positions is performed using the time step $\Delta\tau (= \Delta t/n)$.
4. Steps 2 and 3 are repeated n times.
5. Return to step 1.

The BD is performed using "normal" long time step Δt, while this single step dynamics is composed of n iteration using short time step $\Delta\tau = \Delta t/n$ to relax faster motions into equilibrium. Therefore, when the number of n is increased, computation time is reduced greatly compared to single time step method ($n = 1$), because the computation time required to evaluate the long-range non-bonded interactions, $\mathrm{F_{slow}}$, dominates all other parts of the calculations.

METHODS

United-Atom Model and Force Field

The AMBER91 united-atom force field [25] was used; non-polar hydrogens are included in the carbon

atoms they attach, while polar hydrogens are explicitly treated. Although in the original AMBER91 force field the hydrogen bonding term was just a function of distance, in this simulation distance- and angle-dependent function was used as described in our previous work [22].

In this simulation, we used an implicit solvent model combining two models; solvent-accessible Surface Area (SA) model [26] and Distance-dependent Dielectric (DD) model [27] with $\varepsilon = 3r_{ij}$ where r_{ij} is the distance between atom i and j. In SA model, we used the approximate analytical expression of Hasel *et al.* [28] for solvent-accessible surface area calculation and the atomic solvation parameters determined by Wesson and Eisenberg [29]; $\sigma(\mathrm{C}) = 12\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $\sigma(\mathrm{O,\ N}) = -116\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $\sigma(\mathrm{S}) = -18\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $\sigma(\mathrm{O^-}) = -175\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$ and $\sigma(\mathrm{N^+}) = -186\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, which were derived by fitting to small-molecules' free energies of solvation. In their model, amide, hydroxyl and thiol groups were treated as united-atoms. However, protons of those groups were explicitly treated for solvent-accessible surface area calculation in this study. So, in the first place we used the same atomic solvation parameters for those protons as those for the atoms they attach, respectively, although those parameters need to be optimized in the future.

Dynamics Simulations

To test the efficiency, accuracy and stability of the BD simulation using the multiple time step algorithm, various simulations of a designed ββα folded 28-mer peptide, pda8d (PDB code 1psv, KPYTARIKGRTFSNEKELRDFLETFTGR with 304 atoms) [30], were performed. First, in order to determine the appropriate number of n, we checked the differences of forces (DF) on an atom between j and $j+1$ time steps with various n:

$$DF \equiv \sqrt{\left|\mathbf{F}_j^{n=i} - \mathbf{F}_{j-1}^{n=i}\right|^2}, \quad i = 2, 4, 6, 8, 10. \qquad (4)$$

DF reflects the magnitude of the integration error. For DF evaluations, we considered the long-range non-bonded forces ($\mathrm{F_{slow}}$), DF_{slow}, and the short-range bonded forces ($\mathrm{F_{fast}}$), DF_{fast}, separately. For the calculations of DF_{slow} and DF_{fast}, 1000 iterations of the long time step (Δt) and of the short time step ($\Delta\tau$) were used, respectively. And three structures were used for sampling as mentioned below and DFs were averaged over 1000 iterations, three sample structures and all atoms under the applied forces.

Three structures obtained from the following BD simulation using single time step at 3, 4 and 5 ns were used for the DF evaluations. BD simulation from a NMR solution structure using the multiple time step algorithm for 10 ns was also performed. We used the first model of the NMR solution structures of pda8d solved by Dahiyat *et al.* [30] as the starting structure of the BD simulations. Instead of energy minimization of the peptide, the BD simulation using single time step of 5 fs without random force was performed for 1000 steps. Simulation temperature was 298 K with the viscosity of water, $0.128\,\mathrm{kcal\,mol^{-1}\,\AA^{-3}}$ ps. The Stokes radius of each atom was its van der Waals radius plus 1.4 Å. The LINCS algorithm [17] was used to constrain bond lengths. The adaptive time step method [31] described in the previous paper [22] was also used. In this paper, the time step was split into five smaller ones whenever the angle energy difference between $i-1$th and ith steps became larger than $20\,\mathrm{kcal\,mol^{-1}}$. Cutoff method was not used. For the multiple time step method, short time step ($\Delta\tau$) of 5 fs was used throughout the simulations. This value was sufficient to keep numerical stability even at high temperature (data not shown). Coordinates and energies were recorded every 10 ps during the 10 ns simulation.

For the comparison of the computation time, we also performed a short MD simulation in explicit water molecules. The MD simulation was performed using the MD program AMBER 4.1 [32] with united-atom force field [25], as described in the previous paper [22]. Simulation temperature was the same as used for BD simulation.

Finally, folding simulations of a 13-mer α-helical peptide, peptide III (acetyl-AETAAAKFLRAHA-$\mathrm{NH_2}$ with 125 atoms), from linear state were performed using the BD algorithm with the multiple time step method for three times using different random seeds. Peptide III is an analogue of the C-peptide of ribonuclease A designed by Shoemaker *et al.* [33]. From far-UV circular dichroism spectrum measurements, it is estimated that this peptide contains about 50% helix in 0.1 M NaCl solution, pH 5.2, 276 K [33,34]. Therefore, we used the protonated state of His12 in the simulation. Coordinates and energies were recorded every 100 ps during the simulation. To assign the secondary structure of the peptide generated from BD simulations, we used the program DSSP [35]. For calculating the helix content, we divided the number of helical residues by the total number of residues.

All the simulations were performed on a personal computer with Pentium4 2.5 GHz processor.

RESULTS

ββα Folded 28-mer Peptide (pda8d)

To determine the optimum time step, we checked the magnitudes of integration errors of the non-bonded

TABLE I The average magnitudes of integration errors of the long-range non-bonded terms ($\langle DF_{\mathrm{slow}}\rangle$) in the BD simulations using the multiple time step algorithm with various numbers of n

		$\langle DF_{slow}\rangle$ (kcal mol^{-1} Å^{-1})*			
n	*Long time step Δt (fs)*	*van der Waals*	*Electrostatic*	*Hydrogen bonding*	*Solvation*
1	5	0.24	0.06	0.07	0.03
2	10	0.28	0.07	0.06	0.04
4	20	0.42	0.10	0.08	0.06
6	30	0.48	0.12	0.08	0.06
8	40	0.53	0.13	0.08	0.07
10	50	0.57	0.14	0.09	0.07

* DF_{slow}s were calculated using the Eq. (4), and averaged over 1000 iterations of the long time step Δt, three sample structures and all atoms under the applied forces.

interactions, DF_{slow}, (Table I), and of the bonded interactions, DF_{fast} (Table II), using various numbers of n. Although DF_{slow} increased with the number of n, the values were less than 0.6 kcal mol^{-1} Å^{-1} even $n = 10$ (Table I). For a carbon united-atom (CH_2), which is the most abundant atom in proteins, the magnitude of a random force, $\sqrt{6\zeta k_{\mathrm{B}}T\delta(t)}$, is about 5.6 kcal mol^{-1} Å^{-1} at 298 K using its van der Waals radius 2.235 Å. Therefore, the magnitudes of integration errors of the non-bonded forces are small compared to the magnitude of random force. On the other hand, while DF_{fast} decreased with the number of n, the value of angle term was greater than 1.5 kcal mol^{-1} Å^{-1} with $n = 10$ (Table II), which is large compared to the integration errors of the non-bonded forces and about one-fourth of the magnitude of random force. We will discuss this point in the "Discussion" section. The decrease in DF_{fast} with n indicates that the bond angles, dihedral angles, improper torsion and 1–4 interactions are relaxed at a local structure during n steps with time step of $\Delta\tau$. In other words, for the short-range bonded interactions, the multiple time step algorithm acts like an energy minimization of n steps during Δt under a constant random force.

We also checked the difference of potential energies obtained with multiple time step BD simulations from that of single time step BD simulation ($n = 1$) without random force as micro-canonial simulations, giving very small difference even with $n = 10$ (data not shown). Thus the multiple time step algorithm gives sufficient numerical accuracy.

Table III lists the time required to run 1 ns simulation of pda8d with the multiple time step method. It also lists the relative times compared to BD using single time step ($n = 1$) and MD simulation with explicit water molecules. The computation time of the BD simulation using the multiple time step algorithm with $n = 8$ or 10 was reduced by factor of 6–7 compared to the BD using single time step method. Moreover, the computation time was reduced by a factor of 160–180 compared to the MD simulation.

The above results show that the multiple time step algorithm with $n = 10$ reduce the computation time of the BD simulation greatly without noticeable loss of numerical accuracy. However, BD simulations became unstable in a few cases because of unfavorable atomic clashes when the multiple time step algorithm with $n = 10$ was used. Thus, we used $n = 8$ ($\Delta t = 40$ fs) for the multiple time step algorithm in following simulations.

We performed a dynamics simulation for 10 ns using the multiple time step. Figure 1 shows Cα RMS deviation (RMSD) of the peptide from the NMR structure as a function of time. The BD simulation using the multiple time step algorithm gave stable trajectory over the 10 ns simulation, as judged by the smaller RMSD than 3 Å. Figure 2 shows the radius of gyration (R_g) of the peptide as a function of time. The BD simulation gave stable R_gs at 9.3 Å during 10 ns

TABLE II The average magnitudes of integration errors of the short-range bonded terms ($\langle DF_{\mathrm{fast}}\rangle$) in the BD simulations using the multiple time step algorithm with various numbers of n

		$\langle DF_{fast}\rangle$ (kcal mol^{-1} Å^{-1})*				
n	*Long time step Δt (fs)*	*Bond angles*	*Dihedral angles*	*Improper torsion angles*	*1–4 van der Waals*	*1–4 Electrostatic*
1	5	9.66	1.48	4.73	0.51	0.03
2	10	5.95	0.94	2.81	0.28	0.02
4	20	3.54	0.59	1.56	0.16	0.02
6	30	2.45	0.43	1.01	0.11	0.01
8	40	1.84	0.34	0.73	0.09	0.01
10	50	1.46	0.28	0.57	0.07	0.01

* DF_{fast}s were calculated using the Eq. (4), and averaged over 1000 iterations of the short time step $\Delta\tau$ of 5 fs, three sample structures and all atoms under the applied forces.

TABLE III Computation time required for 1 ns simulation of the ββα folded 28-mer peptide, pda8d

Algorithm	*n*	*Long time step Δt (fs)*	*Time (min)**	*Relative time†*
BD‡	1	5	75.3	1.00 (27.3)
	2	10	46.0	0.61 (44.8)
	4	20	21.8	0.29 (94.4)
	6	30	15.8	0.21 (130.4)
	8	40	12.8	0.17 (160.9)
	10	50	11.1	0.15 (185.0)
MD¶	–	–	2057	27.3 (1.0)

* All calculations were performed using Pentium4 2.5 GHz processor.
† The relative time compared to the time of explicit water MD simulation using cut-off radius of 9 Å is given in parentheses. ‡ The simulations were performed using the BD program with the multiple time step algorithm developed here. The short time step $\Delta\tau$ was 5 fs. All covalent bonds were constrained with LINCS algorithm. Number of atoms was 304.
¶ The simulation was performed using the MD program AMBER with united-atom force field. The peptide was solvated using a box extending at least 10 Å in all directions. All covalent bonds were constrained with SHAKE algorithm. Cut-off radius was 9 Å. The time step was 2 fs. Number of atoms was 7681.

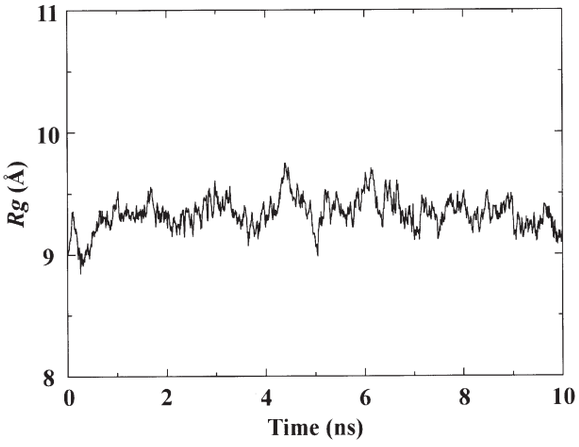

FIGURE 2 Radius of gyration (R_g) of the pda8d peptide obtained from Brownian dynamics simulation as a function of simulation time.

simulation. Those results indicate that the BD using the multiple time step algorithm can provide stable simulations.

α-Helical Peptide (peptide III)

We also performed folding simulations of an α-helical peptide, peptide III, from a linear state using the multiple time step algorithm with $n = 8$. Recent experiments on the helix-coil transition of Ala-based peptides employing laser-induced temperature-jump method have shown that the formation of α-helix occurs in a few nanoseconds [36–41]. In order to simulate the folding, 400 ns BD simulations were performed for three times using different random seeds and we obtained three trajectories. Figure 3 shows the helix contents as a function of time and snapshots of the first trajectory at various periods are shown in Fig. 4. In all the BD simulations, the formations of α-helix were observed and all the trajectories started from a linear state reached the folded state (helix content is about 85%; all residues except for both termini are in helical form) in about 300 ns. Although this folding time obtained from three BD simulations is somewhat arbitrary, the time scale agrees with the folding time determined experimentally. The thermodynamics and kinetics of folding of the α-helix peptide will be examined in detail elsewhere.

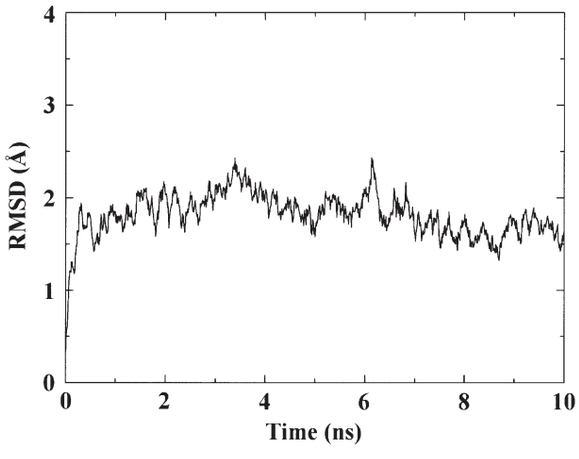

FIGURE 1 Cα RMS deviation from the NMR structure of the pda8d peptide as a function of simulation time.

DISCUSSION

The purpose of this study is to develop the multiple time step algorithm applied to BD simulation that enables simulation of longer time scale motions of biomolecules without significant loss of accuracy. In order to achieve our purpose, the selection of the optimum time step is a critical point. For the BD algorithm, the size of time step is limited by the requirements that (1) the systematic forces should be constant during Δt, and (2) $\gamma\Delta t \gg 1$. The errors in the systematic force per step act as an additional source of random noise, thus raising the effective temperature of the simulation. Therefore, any integration errors must remain much smaller than the magnitude of the random force.

In this study, we used the long time step Δt of 40 fs with $n = 8$. The magnitudes of integration error of the long-range non-bonded terms were sufficiently small compared to the magnitude of the random force (Table I). Thus, the first requirement of the BD algorithm is satisfied. The collision frequency γ of the carbon united-atom calculated from equation (2) is $263\,\mathrm{ps}^{-1}$ with the long time step $\Delta t = 40$ fs, resulting $\gamma\Delta t = 10.5$. Although 10.5 may not be much greater

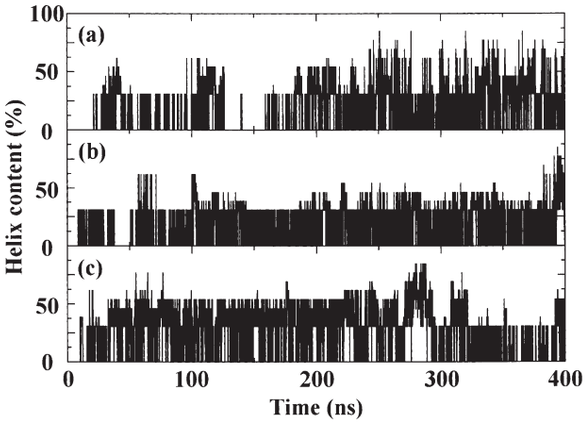

FIGURE 3 Helix contents of the peptide III as a function of simulation time. Helix contents were calculated based on the secondary structure assignment program DSSP [35]. (a) Trajectory 1, (b) trajectory 2 and (c) trajectory 3.

than 1, $\gamma\Delta t$ term affects the BD algorithm through an exponential function ($\exp(-\gamma\Delta t)$). Therefore, the second requirement of BD algorithm is also satisfied in long-range non-bonded terms.

On the other hand, the magnitude of integration error of the angle term during $\Delta\tau(= 5\,\text{fs})$ may not be small enough compared to random force (Table II) with the drawback that the integration of fast motions caused by the short-range bonded interactions does not follows the BD formalism in a strict sense (because random force is updated every long time step Δt but F_{fast} is evaluated every short time step $\Delta\tau = \Delta t/n$). In this respect, bond angle constraints are a possible solution to decrease the integration error of bond angle term and increase the time step. Eastman and Doniach reported a multiple time step algorithm applied to the diffusive Langevin dynamics (Brownian dynamics) using mathematical constraints of bonds and bond angles [42]. However, it is generally accepted that constraint of the bond angles in simulations of conformationally flexible molecules can cause a deleterious effect on the efficiency with which the system explores configurational space [43]. Therefore, we did not utilize the bond angle constraints in our BD simulation, instead we implemented the multiple time step algorithm in our BD method to achieve the energy minimization for short-range bonded terms at a local structure to keep proper configurations.

Furthermore, since the atomistic BD simulations of a tetra-peptide using a single time step of 10 fs reported by Shen *et al.* reproduced reasonably well the experimental results [23] and the BD simulation of the pda8d using the same time step gave stable trajectories as described in our previous paper [22], the integration errors caused by using $\Delta\tau$ of 5 fs should have little effect on the simulations. Although the short time scale ($\Delta\tau$) dynamics may be somehow awkward and inappropriate with respect to the strict BD formalism, our interest is in longer time scale motions and we think this disadvantage is reasonable price to pay for the resulting reduction in computation time.

By applying the multiple time step algorithm to the BD simulation, the computation time was reduced by a factor of 160 compared to a conventional MD simulation using explicit water molecules (Table III). This reduction made it possible to reproduce the folding of an α-helical peptide that took place on a few hundred nanoseconds.

CONCLUSION

To simulate biological processes for long time, we applied the multiple time step algorithm to the atomistic Brownian dynamics simulation.

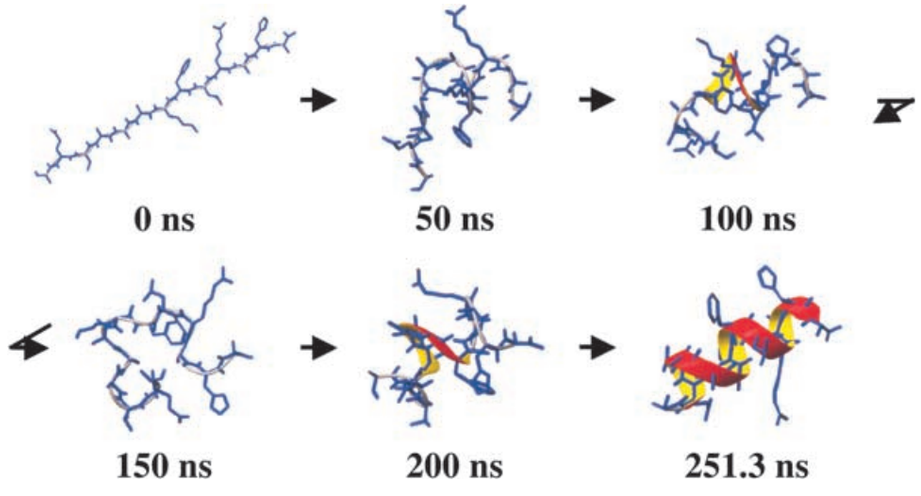

FIGURE 4 Snapshots of peptide III at various periods in Brownian dynamics simulation (trajectory 1; Fig. 3a) using the multiple time step algorithm. The figures are generated with MOLMOL [44].

By using this algorithm, computation time was dramatically reduced and the folding of an α-helical peptide was successfully reproduced, which took place on a few hundred nanoseconds. Numerical stability and accuracy were both satisfied. Since the algorithm can dramatically improve the conformational searching efficiency without noticeable losses of numerical stability and accuracy, it is useful as a powerful tool to study many interesting biological processes.

Acknowledgements

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