Large Scale MD Simulations of Proteins on the Earth Simulator: Quaternary Structural Changes of Hemoglobin

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The purpose of our group is to computationally demonstrate large structural changes of hemoglobin using COSMOS90 which was accelerated on the Earth Simulator by vectorization and parallelization for all subroutines. COSMOS90 can efficiently simulate proteins in the realistic conditions i.e., in water with all degrees of freedom and long-range Coulomb interactions. Hemoglobin consists of four small proteins (subunits α_1, α_2, β_1, and β_2) which associate with each other and locate at four tops of a tetrahedron (Fig. 1). In our previous study (from 2005 to 2006), we carried out a 45-ns molecular dynamics simulation of hemoglobin for an initial X-ray structure (an oxy T-state hemoglobin with PDB code: 1GZX) which is an unstable structure of oxy-hemoglobin. We found the following features for structural changes of hemoglobin (J. Comput. Chem. vol.28, pp1129- 1136, 2007). Dimers $\alpha_1\beta_1$ and $\alpha_2\beta_2$ moved like two stacks of dumbbells. The distance between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) increased by 2Å (7.4 %) in the initial 15 ns and stably fluctuated at the distance with the standard deviation 0.2Å. The relative orientation of the two dimers fluctuated between the initial X-ray angle -100˚ and about -105˚ with intervals of a few tens of nanoseconds. In the present study (from 2007 to Sept. in 2008), we performed a 45-ns MD simulation under the same condition as the previous simulation for a different initial structure (an oxy R-state structure: PDB code 2DN1 which is a stable structure of oxy-hemoglobin). We found that the distance between the two dimmers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) were maintained close to the initial X-ray structure within the fluctuation of 0.2Å in contrast to the previous simulation for the oxy T-state hemoglobin. We concluded that the quaternary structural change of oxy T-state in the previous study was not an artifact caused by a computational instability but a reliable simulation result.

Keywords: Molecular dynamics simulation, Allosteric effect, RMSD, Hemoglobin, Quaternary Structural change

1. Introduction

Molecular dynamics (MD) simulations using high-speed computers become a necessary tool to investigate protein functions and properties because of the following reasons. Proteins are large molecules consisting of thousands of atoms and have complicated structures. They largely change the whole structure even at the room temperature. It is difficult for experimental approaches to observe dynamics processes of such large structural changes.

A hemoglobin molecule can efficiently transfer oxygen molecules from the lungs to the muscles. The binding of an oxygen molecule to a site of hemoglobin enhances additional oxygen bindings on other sites of the hemoglobin. The X-ray crystal studies showed the structural difference between the initial (oxygen-dissociated) and final (oxygen-associated) states (Fig. 1). Hemoglobin consists of four small proteins (subunits α_1, α_2, β_1, and β_2) which associate with each other and locate at four tops of a tetrahedron. Hemoglobin has two different stable structures (oxy R-state and deoxy T-state structures) depending on whether four oxygen molecules bind to the respective sites. The two structures are different from each other in the quaternary structure, i.e., the location of four subunits.

The binding affinity of oxygen molecules to the sites is low for the T-state structure and high for the R-state structure. The cooperative oxygen binding of hemoglobin is explained by the quaternary structural change from T to R induced by the oxygen bindings, as shown in text books of biochemistry (Fig. 1), as follows. The sequential bindings of four oxygen molecules to the four sites change the quaternary structure from low-affinity T to high-affinity R and enhance the oxygen bindings. The hemoglobin hypothesis describes the quaternary structural change by a degree of freedom, i.e., rotation angle between the two dimmers ($\alpha_1\beta_1$

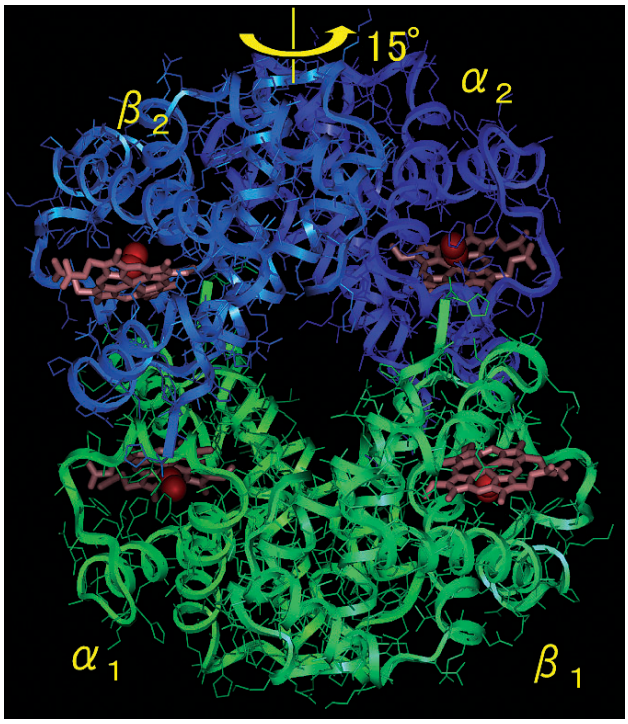

Fig. 1 X-ray structure of hemoglobin. Hemoglobin consists of four small proteins (subunits α_1, α_2, β_1, and β_2) which associate with each other and locate at four tops of a tetrahedron. The structural difference between the oxy and deoxy hemoglobin suggests that the $\alpha_1\beta_1$ dimer rotates against to another dimer $\alpha_2\beta_2$ according to the oxygen binding to four hems.

and $\alpha_2\beta_2$), in the X-ray structures, where the distance between $\alpha_1\beta_1$ and $\alpha_2\beta_2$ is almost identical between the R-state and T-state structures. However, the experimental studies have not yet observed the dynamical process of this quaternary structural change.

The purpose of our study was to perform a long MD simulation as long as possible on the Earth Simulator and to investigate the dynamical features of tertiary and quaternary structures of human adult hemoglobin (HbA) in water without any artificial constraints (Fig. 2). To achieve this purpose, one of the authors (M.S.) accelerated his own software, COSMOS90, by vectorizing and parallelizing it for the Earth Simulator.

2. COSMOS90

COSMOS90 was developed by one of the authors (M.S.) in 1990 and made it possible to simulate a protein in water with all degrees of freedom and with long-range Coulomb interactions using the Particle-Particle and Particle-Cell (PPPC) method[1]. The PPPC method was proposed also by the author to efficiently calculate long-range Coulomb interactions between atomic charges in the order NlogN instead of N^2 by dividing a system into hierarchical cubic cells based on the Barnes & Hut tree code. In 2004, one of the authors (M.S.) tuned up COSMOS90 on the Earth Simulator by vectorizing and parallelizing its all subroutines including the

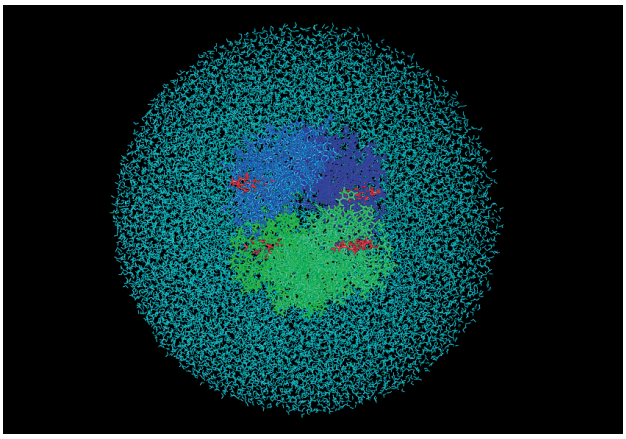

Fig. 2 Human adult hemoglobin (HbA) in a water sphere of radius 66Å. The total number of atoms is 119421.

Barnes-Hut tree construction[2], as follows.

All simulations were performed on the Earth Simulator with COSMOS90. COSMOS90 has a large loop to reiterate the MD time step and advance the simulation time. The loop contains time-consuming subroutines that calculate various forces such as bonded forces (bond, angle, and torsion) and nonbonded forces (Lennard-Jones and Coulomb). All subroutines in this loop were highly vectorized by inserting directive lines to the compiler and parallelized by using the message passing interface (MPI). The parallelization was based on the flat MPI programming; that is, processors inside a node were treated in the same manner as those between nodes.

The calculation of the Coulomb forces is usually the most time-consuming part in MD simulations. In COSMOS90, the Coulomb forces are efficiently calculated by the PPPC method, which utilizes the space subdivision based on the Barnes-Hut tree construction.[3] The Barnes-Hut tree is constructed in parallel by using 8 (or 64) processors independently dividing tree-nodes (that is, cells) of the second (or third) level in the Barnes-Hut tree. In this parallelization, we kept the vector acceleration. All processors make their own interaction tables by searching cells interacting with the atoms of each processor according to the Barnes-Hut tree. Then the interaction table, which is the largest array in COSMOS90, is distributed to all processors and the distribution of the interaction table clears the memory bottleneck that occurs for large-scale simulations.

The performance speed of COSMOS90 was continuously accelerated upon 128 processors of the Earth Simulator. The maximum performance speed for HbA in water was 0.029 s/step for 128 vector processors. The vectorization on a single processor accelerated the performance speed to 12.2 times as fast as the scalar performance. Furthermore, the parallelization on the 128 vector processors accelerated the performance speed to 69 times as fast as the speed with a single vector processor.

3. Four initial X-ray structures

We chose the following four initial X-ray structures to investigate quaternary structural changes of hemoglobin. (1) Oxy T-state structure (unstable structure with PDB code: 1GZX). (2) Oxy R-state structure (stable structure with PDB code: 2DN1). (3) Deoxy T-state structure (stable structure). (4) Deoxy R-state structure (unstable structure).

The first structure (oxy T-state unstable structure) is restricted to the unfavorable T-state quaternary structure probably due to crystal contacts and a low temperature (4°C)[4]. Our MD simulatons can release these restrictions because of the solution environment at the room temperature. The second initial structure (oxy R-state) has the feature of the stable quaternary structure. We performed a long MD simulation (45 ns) for this initial structure in this year to demonstrate the reliability of our simulations. The third initial structure (deoxy T-state structure) was prepared by deoxygenating the unstable oxy T-state hemoglobin with the first initial structure. The fourth initial structure (deoxy R-state structure) was prepared by deoxygenating the stable oxy R-state hemoglobin with the second initial structure.

4. Root Mean Square Deviation (RMSD)

To investigate the structural changes of HbA, we plotted the root-mean-square deviation (RMSD) of main-chain atoms (Cα, C, and N) for the entire HbA molecule (Fig. 3). Dimers α1β1 and α2β2 fitted to their initial X-ray structures had almost the same RMSD values as those of the subunit monomers. In contrast, the unfitted dimers had RMSD values (3.5±0.2Å for α1β1 and 3.4±0.23Å for α2β2) that were substantially larger than the values for the fitted dimers (blue lines vs. black lines in Fig. 3).

The RMSD values for the various dimers indicate the following dynamical features of HbA. The interactions between the subunits within the dimers (that is, between α_1 and β_1 and between α_2 and β_2) were stronger than the interactions between different dimers and thus dimers $\alpha_1\beta_1$ and $\alpha_2\beta_2$ showed almost the same RMSD values as the subunit monomers. Dimers $\alpha_1\beta_1$ and $\alpha_2\beta_2$ changed their relative positions, moving like rigid bodies, and thus the structures of the dimers without fitting deviated greatly from the initial structures.

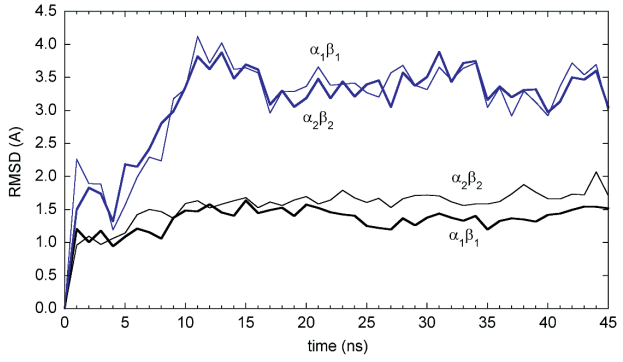

Fig. 3 Root-mean-square deviations (RMSDs) of the main-chain atoms (C_α, C, and N) as a function of time for dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$). The RMSD values were calculated after fitting one of two dimers to the corresponding dimer of the X-ray structure at 1-ns intervals according to the trajectory. Black lines: fitted dimers; Blue lines: dimers without fitting.

5. A model of quaternary structure

We represented the each subunit as a center of mass by neglecting the internal degrees of freedom for the subunits. Then, the quaternary structure of hemoglobin was simply represented by the centers-of-mass model (Fig. 4) with the two parameters, i.e., the distance d_{12} and torsion angle Φ between the dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$). To check the validity of the above centers-of-mass model, we calculated the RMSD of the unfitted $\alpha_2\beta_2$ dimer based on the model (Fig. 5). The

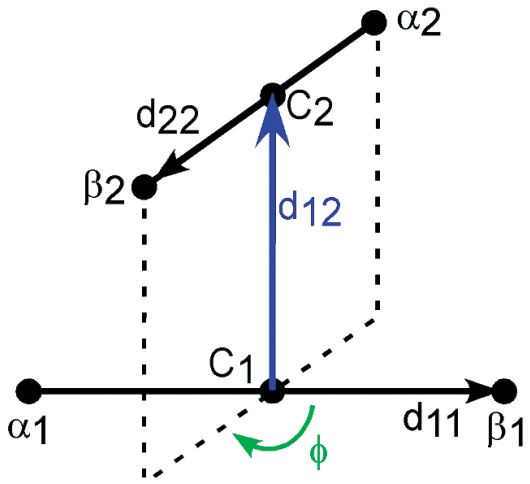

Fig. 4 A model of hemoglobin. Each subunit was presented by the centers of mass. A distance between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) is defined by the distance d_{12} between their geometric centers, C_1 of $\alpha_1\beta_1$ and C_2 of $\alpha_2\beta_2$. A relative orientation of the two dimers is defined by the dihedral angle Φ.

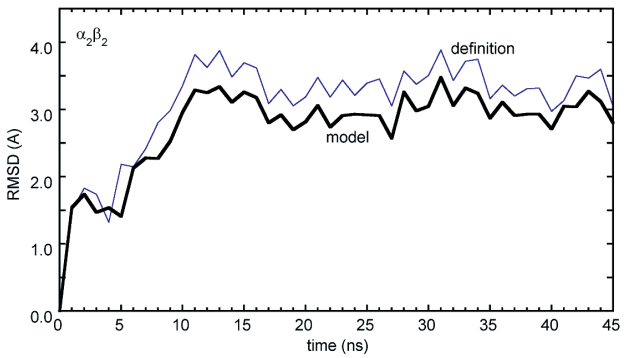

Fig. 5 The RMSD of the unfitted $\alpha_2\beta_2$ dimer was estimated using the model (Fig. 4). The black line denotes the RMSD of the centers of mass with the RMSD of the monomer. The blue line denotes the RMSD obtained in Fig. 3.

RMSD of the unfitted $\alpha_2\beta_2$ dimer was simply estimated from the displacements of the centers of mass for α_2 and β_2 from their initial X-ray positions (black line in Fig. 5), where the three points (α1, β1, and C2) were fitted to their initial X-ray positions. The RMSD obtained as a function of time (black line) was almost the same as that of the real HbA (blue line). This result means that the RMSD of the unfitted $\alpha_2\beta_2$ dimer in Fig. 3 was well described by the relative motion of the centers of mass for α_2 and β_2. In other words, this model is reasonable to describe the quaternary dynamics of hemoglobin.

6. Oxy T-state structure

Since the initial structure used in the previous simulations, oxy T-state structure, is an unstable structure of the oxy-hemoglobin, some structural changes from the T to R state are expected for a very long simulation. Soaking experiments for oxygen bindings to hemoglobin in the crystal environment usually break the crystals probably because of the large structural changes of hemoglobin, which break favorable inter-molecular interactions stabilizing the crystals. However, the crystal of 1GZX (the previous initial structure) was not broken by the soaking experiments. Quaternary structure was maintained to the T-state in spite of the oxygen bindings to hemoglobin, as described in the article[4]. The authors of the article explained the result by the strong crystal contacts of molecules and a low temperature environment (4˚C). Our simulations in water and at the room temperature released these restrictions and then allow the structural change from T to R state.

We performed a 45-ns MD simulation of HbA in water with all degrees of freedom (including bond stretching) and with long-range Coulomb interactions. The 45-ns simulation of this study does not reach the order of μs but is 22 times as longer as the present longest simulation (2 ns).[5] The distance between the two dimmers (d_{12}) and their relative rotation angle Φ were monitored according to the time (Fig. 6). The distance between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) increased by 2Å (7.4 %) in the initial 15 ns and stably fluctuated at the distance with the standard deviation 0.2Å. The relative orientation of the two dimers fluctuated between the initial X-ray angle -100˚ and about -105˚ with intervals of a few tens of nanoseconds.

7. Oxy R-state structure

To deny another possibility that some computational artifacts unstabilize the initial X-ray structure of oxy T-state. We planned to perform an additional simulation from the different initial structure (oxy R-state: PDB code 2DN1) which is the stable structure of oxy-hemoglobin. It is expected that our simulation maintains the X-ray quaternary structure of hemoglobin because this structure do not have any stresses.

The distance between two dimmers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) was plotted as a function of time (Fig. 7). This figure showed that the quaternary structure of oxy R-state hemoglobin was maintained close to the initial X-ray structure during 45 ns in contrast to the oxy T-state structure.

8. Other structures

We prepared other initial structures (deoxy T-state and dexoy R-state). However, we did not perform MD simulations from these initial structures because the CPU time was expired on Sept. in 2008.

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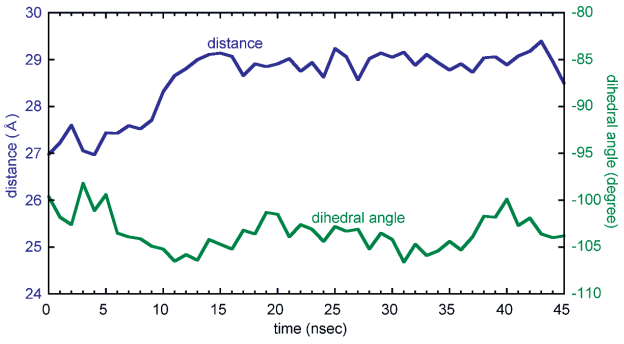

Fig. 6 The quaternary structure parameters (defined by Fig. 4) as a function of time. The blue line denotes the distance d_{12} between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) for the simulation started from the oxy T-state structure. The green line denotes the relative orientation Φ of the two dimers for the same simulation.

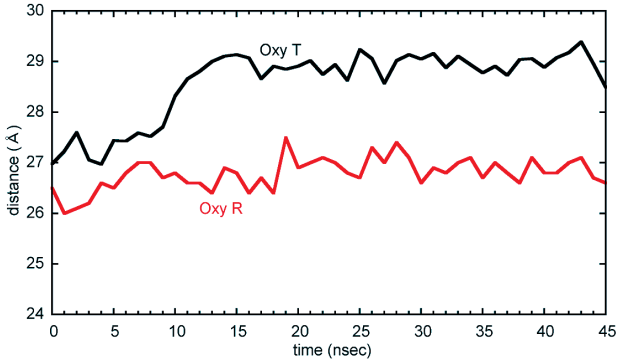

Fig. 7 The quaternary structure parameters (defined by Fig. 4) as a function of time. The red line denotes the distance d_{12} between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) for the simulation started from the oxy R-state structure.

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地球シミュレータによる蛋白質の大規模シミュレーション：ヘモグロビンの高次構造変化

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我々のグループの目的は、ヘモグロビンの大きな立体構造変化（四次構造変化）の仕組みを、独自に開発したソフトウエアCOSMOS90と地球シミュレータを用いて、分子動力学シミュレーションによって明らかにすることである。COSMOS90は、開発者の斎藤によって地球シミュレータ上でベクトル化と並列化とを行って高速化している。COSMOS90によって、ヘモグロビンを全原子、全自由度、全相互作用を考慮して、リアルな条件下でシミュレーションを長時間行った。これまでに、ヘモグロビンに対してこのようなアプローチはなかった。まず、我々は、酸素結合型不安定構造（oxy T-state）のヘモグロビンを水中に置き、45 nsecにわたってシミュレーションを行った。その結果、我々は以下のように、ヘモグロビンを構成するサブユニット間の構造変化を観察することに成功した。二つのサブユニット$\alpha_1\beta_1$と$\alpha_2\beta_2$は、相対的な距離が2Å離れた。また、互いの回転角方向のゆっくりした揺らぎが観測できた。我々のシミュレーションで観測した四次構造変化が、計算上のartifactでないことを確認するために、安定な四次構造を持つ酸素結合型安定構造（oxy R-state）ヘモグロビンについて、全く同じ条件下で、シミュレーションを行った。その結果、oxy T-stateの結果と対照的に、ヘモグロビンの四次構造は長時間安定に保たれた。したがって、我々のシミュレーションで明らかになったoxy T-stateヘモグロビンの新たな四次構造変化は、実際に溶液中で起こりうるリアルな現象であると確信した。

キーワード：分子動力学シミュレーション, アロステリック効果, RMSD, ヘモグロビン, 高次構造変化