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

Project Representative
Minoru Saito Graduate School of Science and Technology, Hirosaki University

Authors
Minoru Saito *1, Isao Okazaki *1 and Akito Taneda *1
*1 The Graduate School of Science and Technology, Hirosaki University

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 simulations of hemoglobin for the initial X-ray structure (an oxy T-state hemoglobin with PDB code: 1GZX) which is an unstable structure of oxy-hemoglobin (J. Comput. Chem. vol.28, pp1129- 1136, 2007). We found the following features for the structural changes of hemoglobin. Dimers $\alpha_1\beta_1$ and $\alpha_2\beta_2$ maintained structures close to their respective X-ray structures while they moved relative to each other like two stacks of dumbbells. The distance between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) increased by 2 Å (7.4%) in the initial 15 ns and stably fluctuated at the distance with the standard deviation 0.2 Å. 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 (2007), we performed a 15-ns MD simulation for a different initial structure (an oxy R-state structure: PDB code 2DN1) which is the stable structure of oxy-hemoglobin under the same condition as the previous simulations. We found that the distance between two dimmers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) were maintained close to the initial X-ray structure within the fluctuation of 0.2 Å in contrast to the previous simulations for the oxy T-state hemoglobin.

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

1. Introduction

Molecular dynamics (MD) simulation 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. Furthermore, they largely fluctuate and easily change the whole structure even at the room temperature.

A hemoglobin molecule can efficiently transfer oxygen molecules from the lungs to the muscles. The binding of an oxygen molecule to a site enhances additional oxygen bindings on other sites of a hemoglobin. Various experimental studies revealed that this cooperative binding is associated with a large structural change. The X-ray crystal studies showed the structural difference between the initial and final states (Fig. 1). However, the experimental studies have not yet observed the dynamical process of the structural change.

The purpose of our study was to perform a long MD simulation as long as possible on one of the fastest supercomputers in the world 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, which was the fastest supercomputer in the world during the period from 2002 to 2004. 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.

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

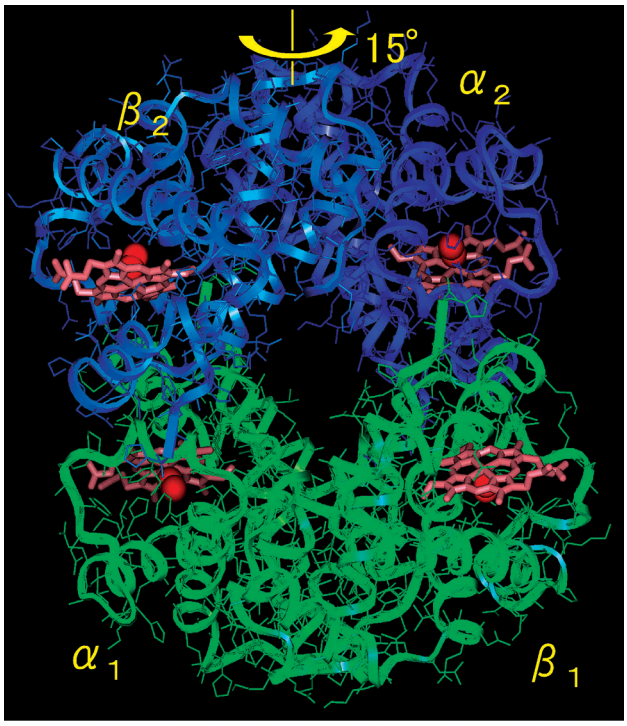

Fig. 1 X-ray structure of hemoglobin. Hemoglobin consists of four small proteins (subunits α_1, α_2, β_1, and β_2) which associate with each other and locate at four tops of a tetrahedron. The structural difference between the oxy and deoxy hemoglobin suggests that the $\alpha_1\beta_1$ dimer rotates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

(M.S.) tuned up COSMOS90 on the Earth Simulator by vectorizing and parallelizing its all subprocesses including the Barnes-Hut tree construction[2].

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 was constructed in parallel and in keeping with the vector acceleration by using 8 (or 64) processors independently dividing nodes (that is, cells) of the second (or third) level in the tree, respectively. All processors made their own interaction tables by searching cells interacting with the atoms of each

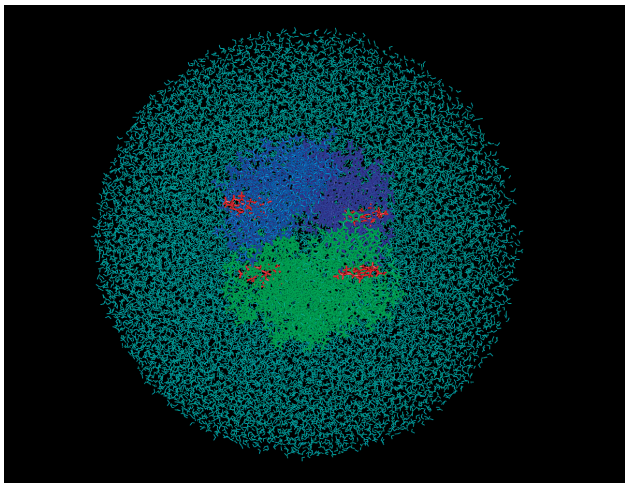

Fig. 2 Human adult hemoglobin (HbA) in a water sphere of radius 66 Å. The total number of atoms is 119421.

processor according to the Barnes-Hut tree. Then the interaction table, which is the largest array in COSMOS90, was 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. Initial X-ray structure

As an initial X-ray structure, we chose the oxy T-state HbA (PDB code: 1GZX) because it was restricted to the unfavorable T-state structure probably due to crystal contacts and a low temperature (4˚C)[4]. Since these restrictions do not exist for the MD simulation in the solution environment at the room temperature (Fig. 2), a very long MD simulation (order of μs) is expected to demonstrate the quaternary transition from the T to R structure. 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].

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 (C_α, C, and N) for the entire HbA molecule (Fig. 3). Dimers $\alpha_1\beta_1$ and $\alpha_2\beta_2$ fitted to the initial X-ray structure had almost the same RMSD values as those of the subunit monomers. In contrast, the unfitted dimers had RMSD values (3.5±0.2 Å for $\alpha_1\beta_1$ and 3.4±0.23 Å for $\alpha_2\beta_2$) that were

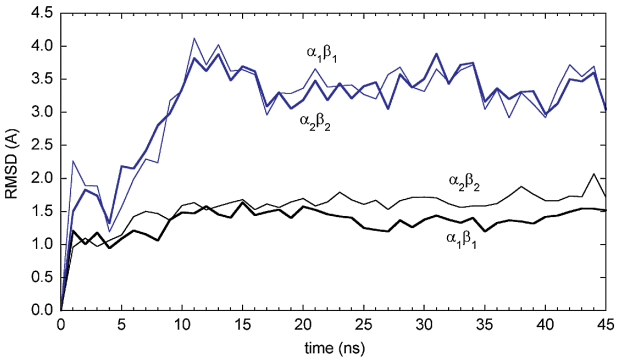

Fig. 3 Root-mean-square deviations (RMSDs) of the main-chain atoms (C_α, C, and N) as a function of time for dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$). The RMSD values were calculated after fitting one of two dimers to the corresponding dimer of the X-ray structure at 1-ns intervals according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

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 of the dimers (that is, between α_1 and β_1 and between α_2 and β_2) were stronger than the interactions between subunits of 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.

5. Dynamics 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 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 (α_1, β_1, and C_2) were fitted to their initial X-ray positions. The RMSD obtained as a function of time (blue line in Fig. 5) was almost the same as that of the real HbA (blue line in Fig. 3). This result means that the RMSD of the unfitted $\alpha_2\beta_2$ dimer in Fig. 5 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. The distance between the two dimmers (d_{12}) and their relative rotation angle Φ were monitored according to the time (Fig. 6). The

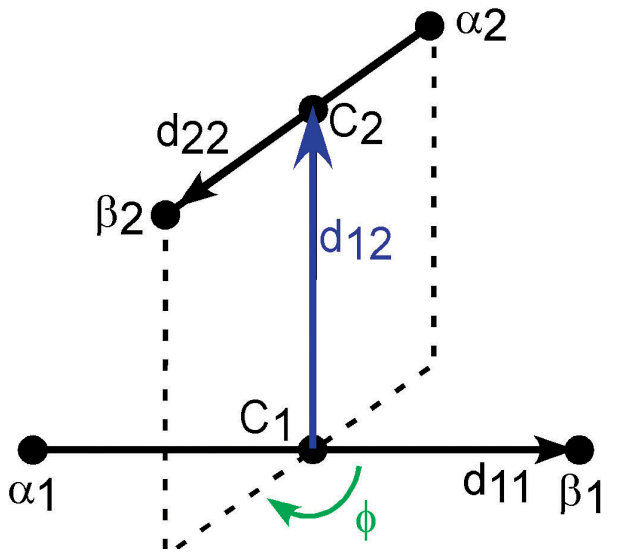

Fig. 4 A model of hemoglobin. Each subunit was presented by the centers of mass. A distance between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) is defined by the distance d_{12} between their geometric centers, C_1 of $\alpha_1\beta_1$ and C_2 of $\alpha_2\beta_2$. A relative orientation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

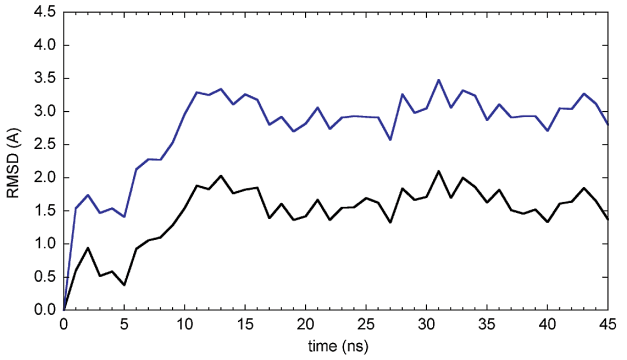

Fig. 5 The RMSD of the unfitted $\alpha_2\beta_2$ dimer was estimated using the model (Fig. 4). The black line denotes the RMSD of the centers of mass for α_2 and β_2. The blue line denotes the RMSD of the centers of mass with the RMSD of the monomer.

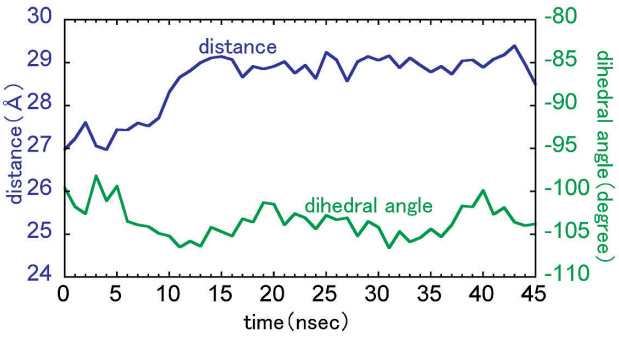

Fig. 6 The quaternary structure parameters (defined by Fig. 4) as a function of time. The blue line denotes the distance d_{12} between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) for the simulation started from the oxy T-state structure. The green line denotes the relative orientation Φ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

distance between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) increased by 2 Å (7.4 %) in the initial 15 ns and stably fluctuated at the distance with the standard deviation 0.2 Å. 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.

6. Stability of quaternary structure

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 structures, 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. In other words, 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. This hemoglobin hypothesis describes the quaternary structural change by a degree of freedom, i.e., rotation angle between the two dimmers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$), as shown in text books of biochemistry (Fig. 1), because the distance between $\alpha_1\beta_1$ and $\alpha_2\beta_2$ is almost identical between the R-state and 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 to hemoglobin in the crystal environment usually break the crystal probably because of the large structural changes of hemoglobin, which break favorable inter-molecular interactions stabilizing the crystal. However, the crystal of 1GZX (the previous initial structure) was not broken by the soaking experiments. The 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.

However, we do not have a proof to deny another possibility that some computational artifacts unstabilize the initial X-ray structure. 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 additional simulation maintains the X-ray quaternary structure of hemoglobin because this structure do not have large suppression.

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 15 ns in contrast to the oxy T-state structure. We have a plan to extend this simulation to 45 ns in 2008.

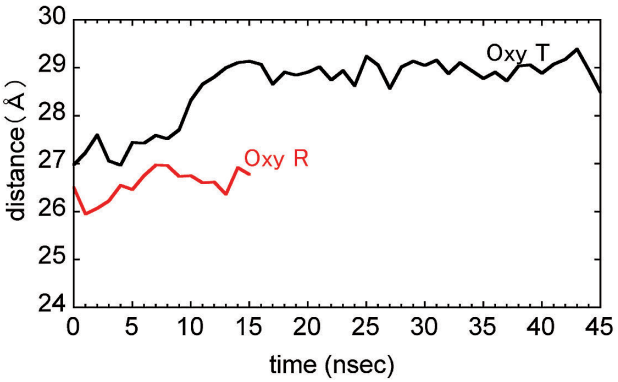

Fig. 7 The quaternary structure parameters (defined by Fig. 4) as a function of time. The red line denotes the distance d_{12} between the two dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) for the simulation started from the oxy R-state structure.

References

[1] M. Saito: Molecular dynamics simulations of proteins in water without the truncation of long-range Coulomb interactions, *Molecular Simulation*, vol.8, pp.321–333 (1992).

[2] M. Saito: Large Scale Simulations of Proteins on the Earth Simulator: Acceleration Performance by Vectorization and Parallelization, *IPSJ Transactions on Advanced Computing Systems*, vol.46, No.SIG 7 (ACS 10), pp.9–17 (2005).

[3] Barnes, J. and Hut, P.: A hierarchical O(NlogN) force-calculation algorithm, *Nature*, vol.324, pp.446–449 (1986).

[4] Paoli, M.; Liddington, R.; Tame, J.; Wilkinson, A.; Dodson, G. Crystal structure of T state hemoglobin with oxygen bound at all four hems. *J Mol Biol*, vol.256, pp.775–792 (1996).

[5] Kovesi, I.; Schay, G.; Yonetani, T.; Laberge, M.; Fidy, J. High pressure reveals that the stability of interdimeric contacts in the R- and T-state of HbA is influenced by allosteric effectors: Insights from computational simulations. *Biochimica et Biophysica Acta*, vol.1764, 516–521 (2006).

[6] Saito, M.; Okazaki, I. A 45-ns molecular dynamics simulation of hemoglobin in water by vectorizing and parallelizing COSMOS90 on the Earth Simulator: dynamics of tertiary and quaternary structures. *J.Comput.Chem.* vol.28, pp.1129–1136, (2007).

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

プロジェクト責任者
斎藤　　稔　　弘前大学大学院　理工学研究科

著者
斎藤　　稔*1，岡崎　　功*1，種田　晃人*1
*1　弘前大学大学院　理工学研究科

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、シミュレーション（MD simulation）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研究は行われていなかった。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。また、一方、酸素結合型安定構造（oxy R-state）のヘモグロビンに対して、同様の条件下で15 nsecのシミュレーションを行った。酸素結合型不安定構造の結果と対照的に、ヘモグロビンの四次構造は、安定に保たれた。

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