Analysis of Global Ecosystem Ecology by Fragment Molecular Orbital (FMO) Method – Analyses of the interactions between virus hemagglutinins and their receptors –

Project Representative
Tadashi Maruyama Center for Marine Biosciences, Japan Agency for Marine-Earth Science and Technology

Authors
Yasuhiro Shimane Center for Marine Biosciences, Japan Agency for Marine-Earth Science and Technology
Kazue Ohishi Faculty of Engineering, Tokyo Polytechnic University
Misako Iwasawa Center for Earth Information Science and Technology, Japan Agency for Marine-Earth Science and Technology
Yuji Hatada Center for Marine Biosciences, Japan Agency for Marine-Earth Science and Technology
Yoshihiro Takaki Department of Subsurface Geobiological Analysis and Research, Japan Agency for Marine-Earth Science and Technology
Takao Yoshida Department of Marine Biodiversity Research, Japan Agency for Marine-Earth Science and Technology
Shigenori Tanaka Department of Computational Science, Kobe University
Satoshi Anzaki Department of Computational Science, Kobe University
Yuto Komeiji Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology
Yuji Mochizuki Department of Chemistry, Rikkyo University
Kaori Fukuzawa School of Dentistry at Matsudo, Nihon University

Continuing from the last year, we analyzed the interaction between morbillivirus hemagglutinin (HA) and host receptor, signaling lymphocyte activating molecule (SLAM), by using the Earth Simulator of JAMSTEC and fragment molecular orbital (FMO) method. In the last year, although we found that the FMO method is useful, the estimations of the three-dimensional (3D) structure of the SLAM and HA were problematic. This year we used the crystal structure of the complex between measles virus (MV) HA and monkey SLAM as a template, we refined the molecular dynamic method to estimate the 3D structures of the complex of HA and SLAMs. The obtained interaction energies between the measles virus HA and marmoset or human SLAM were much more improved.

Keywords: morbillivirus, hemagglutinin, signaling lymphocyte activating molecule(SLAM), fragment molecular orbital (FMO) method, molecular dynamics (MD) method

1. Introduction

The environmental changes caused by climate change and/or human activities are thought to have various impacts on global ecosystem. Those changes may affect the migration profiles and distributions of wild animals including marine mammals and may produce chances for a new contact of the previously separated species/populations. Such changes may affect disease transmission from a population or a species to another, which is virgin to the disease, and thus may cause severe damage to the population/species.

Morbilliviruses (MoV) belonging to the genus *Morbillivirus* in family Paramyxoviridae, have a genome consisting of negative-sense single strand RNAs. They are known to have high host specificity and cause severe diseases in mammals, e.g. measles (human and monkey), canine distemper (dog) and rinderpest (cattle) on land biosphere, and also known to infect marine mammals[1]. Currently, 2 species of morbilliviruses have been reported from marine mammals; phocine distemper

virus (PDV) from seals and cetacean morbillivirus (CMV) from whales[1].

Signaling lymphocyte activating molecule (SLAM; CD150) is the host receptor for MoVs [2]. SLAM is a member of SLAM family proteins (immunoglobulin superfamily), and its variable region domain (SLAM-V) is the interface for MoVs. As cellular receptor of the virus plays an important role for the virus-host specificity, we conducted comparative analyses of interaction energy between the HAs and SLAM-Vs on the host animal cells as reported previously[3]. In the present study, we have estimated structural and energetic dynamisms of the complex of the HA and SLAM by constructing 3D models and calculated the interaction energy by using the fragment molecular orbital method and the Earth Simulator.

2. Construction of docking model

Docking structures of MoV hemagglutinins (MoV-HA) and their receptors (SLAMs) were constructed by homology modeling as described before[3]. Crystal structure PDB 3ALX of the complex of measles virus hemagglutinin (MV-L482R) and marmoset SLAM V domain (marSLAM-V)[4] was used as the template. Total of 13 sets of models were built for this study : MV L482R (PDB 3ALX) - Marmoset SLAM (PDB 3ALX), MV L482R - Human (NM_003037) SLAM, MV L482R - Dog (AF325357) SLAM, MV L482R - Spotted seal (AB428368) SLAM, MV wild (AB012948) - Marmoset SLAM, MV wild - Human SLAM, MV wild - Chimera mouse (NM_013730) /60h/61h SLAM, MV wild - Chimera mouse/60h/61h/63h SLAM, MV wild - Mouse SLAM, CDV (AF164967) - Dog SLAM, CDV - Mouse SLAM, CDV - Human SLAM, and CDV - Chimera mouse/63h SLAM.

3. Conformational sampling using molecular dynamics simulations

Before molecular dynamics (MD) simulations, the protein complexes were solvated by TIP3P water molecules extending 20 Å in all dimensions around the protein, using the Solvate plug-in of VMD program[5]. Total charge was neutralized with 150 mM NaCl using Autoionize plug-in. A typical ionized model contains 527, 30562, 86 and 87 molecules of amino acids, H_2O, Na^+ and Cl^-, respectively (Fig. 1). The total number of 10^5 atoms molecular dynamics simulations were performed on the JAMSTEC Super Computer System (SGI ICE X) using the scalable molecular dynamics software NAMD[6] equipped with CHARMM27 parameter set for protein[7, 8], ions with TIP3P water. All simulations were performed at NVT ensemble (constant number of particles, constant volume and constant temperature) achieved by using the Langevin equation. The Langevin piston Nose-Hoover method[9, 10] was employed to maintain the pressure at 1 atm. Assuming periodic boundary conditions, the particle mesh Ewald (PME) method[11] with a grid spacing of 1.0 Å in each dimension was employed for the computation of long-range electrostatic forces. All simulations employed time steps of 1 fs, 1 fs, and 2 fs for bonded, non-bonded, and full electrostatic calculations, respectively. An energy minimization removing potential problems are done in the first 300 ps. Temperature was risen to 300K with a rising rate of 0.001 K/fs, then kept during duration step of 4.7 ns.

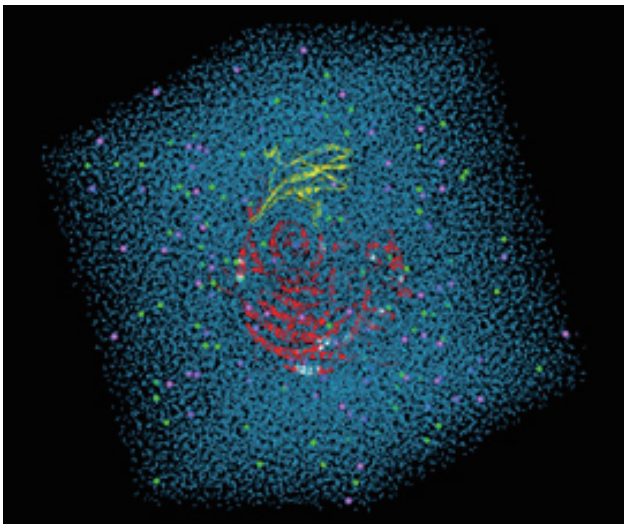

Fig. 1 Measles virus hemagglutinin (red ribbon) and Marmoset SLAM V domain (yellow ribbon) complex in water neutralized with 150 mM NaCl (purple and green spheres). The cell size is 96×97×111Å and is containing total number of 100156 atoms.

4. FMO analyses

Interaction energy between morbillivirus HA and SLAMs were estimated as a total of the inter-fragment interaction energy (IFIE) calculated in the fragment molecular orbital (FMO) method using ABINIT-MP[12, 13] on the Earth simulator. All calculations in our study were performed at the second-body FMO (FMO2) method with the second-order Møller-Plesset perturbation theory (MP2) and reasonable basis set 6-31G. In the first stage of FMO calculation, the protein models were fragmented into single amino acid with the exception of the disulfide-bond residues.

5. Results

We examined 13 data set in combinations of 3 types of MoV-HA (MV-L482R, MV-Wild, CDV) and 8 types of SLAMs (marmoset, human, dog, spotted seal, mouse, chimera mouse/60h/61h, chimera mouse/60h/61h/63h, chimera mouse/63h) in this study. A total of 5.6 ns MD simulations were run on 128 processes that required 258 GB of memory and 2624 CPU hours for a typical dataset. Figure 2 shows fluctuations of physical parameters (total energy, pressure, temperature and volume) vs the duration, indicating that our simulation was successfully controlled.

After the MD simulations, protein structures extracted from trajectories were selected for representatives around the time, and subjected to calculate FMO-IFIE using ABINIT-MP on the

Earth simulator. In the case of MV-L482R HA and Marmoset SLAM V complex derived from the crystal structure, the complex model consisted of 522 fragments in 528 amino acids, 8297 atoms and 31598 electrons, requiring 144.2 CPU hours (when using 8 nodes, 64 processors) and 72 GB (9.0 GB/node) of memory.

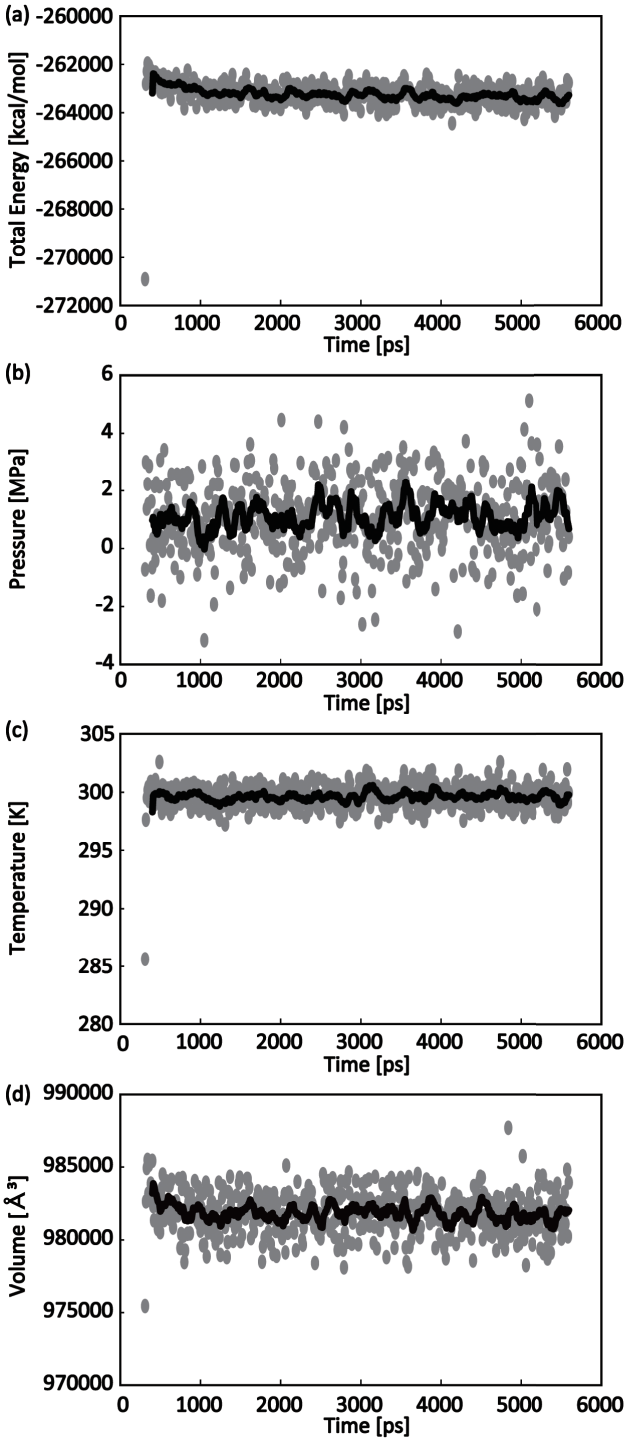

Fig. 2 Results of molecular dynamics simulation. The system contained the complex of Measles virus hemagglutinin (MV-L482R HA) and Marmoset SLAM V domain. (a), change of total energy; (b), change of pressure; (c) change of temperature; (d), change of volume. (a)-(d), dots and line indicates the raw data and the moving average of 10 ps during the warm-up to the equilibrium period (300-5600 ps).

Figure 3 shows the dynamics of interaction energy between MV-L482R HA and Marmoset SLAM V with root-mean-square deviation (RMSD) of main-chain atoms (Cα, C and N) for the entire model. The most stable conformer was found at 4.6 ns in the equilibrating period with -885.5 kcal/mol of interaction energy. It was lower than the hypothetical minimum energy of -870.1 kcal/mol estimated from the minimum energy structure in water derived from the crystal structure (at 300 ps in Fig. 2). RMSD value was increased consistently from the initial structure to an equilibrium point, 0 to 3.5 angstrom, detecting a small conformational change on the complex. The result suggested that FMO analysis following MD simulation could support the refinement of protein-protein interaction and the estimation of native interfaces.

As described above the MD simulation based on a crystal structure gave a reasonable coordinates, whereas remaining other 12 datasets made from the homology models did not. In fact, RMSD and interaction energy became progressively worse during the course of the simulations. The problem may be caused by inaccurate estimations of the initial coordinates. While significant progress has been accomplished in accurate docking of small molecules[14, 15, 16, 17], this protein-protein docking technique is still very challenging due to the high cost of sampling the enormous conformational space. It is not only an important technique for understanding an interaction between virus and host receptor system, but also the key to theoretical drug design. We will address this problem in our future work.

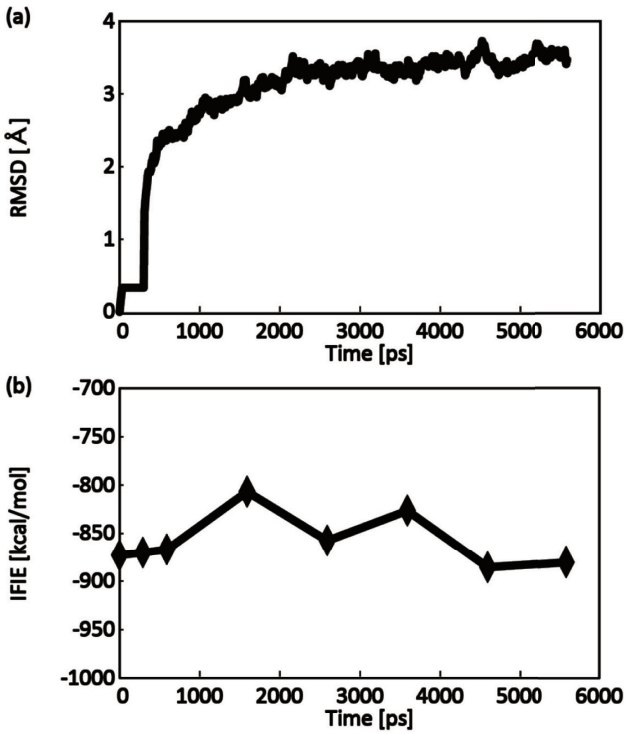

Fig. 3 Dynamisms of Measles virus hemagglutinin (MV-L482R HA) and Marmoset SLAM V domain during 5.6 ns of MD simulation. (a) shows backbone RMSD. (b) indicates IFIE energies between the molecules.

Acknowledgement

The Earth Simulator Version of ABINIT-MP was provided by Center for Research on Innovative Simulation Software in The University of Tokyo and NEC Corporation. The authors would like to thank all members of the Center for Earth Information Science and Technology (CEIST).

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フラグメント分子軌道法の地球生態系解析への応用
－モルビリウイルスヘマグルチニンと受容体の相互作用の解析－

課題責任者
丸山　　正　　海洋研究開発機構　海洋生命理工学研究開発センター
著者
嶋根　康弘　　海洋研究開発機構　海洋生命理工学研究開発センター
大石　和恵　　東京工芸大学　工学部
岩沢美佐子　　海洋研究開発機構　地球情報基盤センター
秦田　勇二　　海洋研究開発機構　海洋生命理工学研究開発センター
高木　善弘　　海洋研究開発機構　深海・地殻内生物圏研究分野
吉田　尊雄　　海洋研究開発機構　海洋生物多様性研究分野
田中　成典　　神戸大学大学院　システム情報学研究科
安崎　　聡　　神戸大学大学院　システム情報学研究科
古明地勇人　　産業技術総合研究所　バイオメディカル研究部門
望月　祐志　　立教大学　理学部
福澤　　薫　　日本大学　松戸歯学部

気候変動や人類活動による地球環境の変化は、しばしば生物の生態に大きなインパクトを与えて地球規模での生息域の変化を引き起こす。ウイルスや病原性微生物などの病原体や、それらの媒介生物の生息域の拡大は、これまで時間・空間的な隔絶により出会う事のなかった生物種との接触の機会を与え、免疫を持たない新たな宿主生物との間で爆発的な感染を引き起こす可能性がある。とりわけ海棲生物の場合には移動域が広く、海水によるウイルスの拡散が制御や対策を困難とする。

モルビリウイルス属はパラミクソウイルス科に属するRNAウイルスであり、ヒト麻疹（MV: Measles virus）や牛疫（RPV: Rinderpest virus）、犬ジステンパー（CDV: Canine distemper virus）など、感染力が強く、劇症化しやすい特徴がある。大西洋沿岸地域においてはアザラシ（PDV: Phocine distemper virus）、クジラやイルカ（CMV: Cetacean morbillivirus）などの海洋性ほ乳類の大量死が度々問題になっている。現在のところ太平洋側での感染は確認されていないが、CDV、PDV、CMVは血清学的に相互に感染性を示すことから、全球規模での感染拡大に備えたリスク評価や薬剤開発が求められる。

我々はモルビリウイルス感染の分子機構を理解するために、種々のウイルスのエンベロープタンパク質ヘマグルチニンと宿主受容体Signaling lymphocyte activating molecule（SLAM）との相互作用をフラグメント分子軌道（FMO）法により解析した。複合体構造の最適化と動的エネルギー変化の解析を目的として、分子動力学法でサンプリングした構造から2分子間の相互作用エネルギーを求めた。X線回折により結晶構造が明らかになっているヒト麻疹ウイルスと小型サルのSLAMでは、水和後の極小構造で-870kcal/mol、300 Kで1 nsの振動後に最小値となる-807kcal/molを示した。続く4 nsの振動では1 nsごとに-858 kcal/mol、-826 kcal/mol、-886 kcal/mol、-880 kcal/molと変化し、RMSDは3.5付近に収束した。時間経過と共に2分子間の相互作用は安定化する傾向を示しており、分子配座は初期構造よりも最適化されているが、構造の平衡化にはより長時間の分子動力学計算が必要と示唆された。一方、ホモロジーモデルを初期構造としたデータセットでは、分子動力学計算中に2分子の解離による相互作用エネルギーの急激な減少が観測されており、初期構造の構築方法に課題がある事が示唆された。

キーワード：モルビリウイルス，ヘマグルチニン，signaling lymphocyte activating molecule（SLAM），
フラグメント分子軌道（FMO）法，分子動力学（MD）法