Large-Scale Electronic-State Calculations of Influenza Viral Proteins with Fragment Molecular Orbital Method and Applications to Mutation Prediction

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On the basis of the fragment molecular orbital (FMO) method, we performed the FMO-MP2 and FMO-MP3 electronic-state calculations on the Earth Simulator (ES2) for a protein complex consisting of HA (hemagglutinin) trimer and two Fab fragments. The FMO-MP3/6-31G calculation, which might be the world's largest target system (36160 atoms) for correlated all-electron calculations to date, was completed in 5.8 hours with 128 nodes. Thus, a realistic applicability of FMO-MP3 calculations to large-scale proteins has been demonstrated with the ES2 system. Molecular interaction analyses based on the FMO-MP2.5 results were then carried out for the prediction of probable mutations in HA associated with the escape from antibody pressure.

Keywords: FMO (fragment molecular orbital) method, MP (Moeller-Plesset) perturbation theory, influenza virus, HA (hemagglutinin)

1. Introduction

Influenza is one of the most important infectious diseases of humans. Recent concerns about the avian and swine influenza viruses highlight its threat and the need to understand its evolutionary dynamics. The influenza virus has a remarkable ability to escape host defense mechanisms by altering its binding characters through changes of amino acid residues in the pertinent proteins. This property is referred to as antigenic drift and has been thought to result from the accumulation of a series of amino acid changes (mutations) in antigenically important regions of proteins. In addition, the viral resistance against some drugs is associated with analogous mutation properties as well. It is thus essential to elucidate the molecular mechanisms by which viruses alter their ligand binding characters in order to find an efficient way to prepare for the pandemics and epidemics of influenza.

There are two types of well-known proteins on the surface of influenza virus. One is hemagglutinin (HA) associated with the infection into host cells. Another is neuraminidase (NA) involved in the escape from infected cells. To investigate the interactions between these proteins and their binding partners at the molecular level, we need to resort to some theoretical methods in computational chemistry. Considering the accuracy in molecular simulations, ab initio quantum-chemical approaches would be most dependable for the computational analysis of molecular interactions, whereas these kinds of simulations would demand huge amount of computer resources for biomolecular systems. Here, we employ the fragment molecular orbital (FMO) method [1], which has been developed for efficient and accurate ab initio calculations for biomolecules, for the detailed analysis of molecular interactions in HA and NA systems. In this context, we pay attention to the inclusion of electron correlation effects in terms of Moeller-Plesset (MP) type perturbative treatments in order to appropriately describe the weak interactions such as dispersion forces between hydrophobic residues.

In 2008, we performed [2] the FMO-MP2/6-31G calculation for an antigen-antibody system consisting of the HA monomer and the Fab fragment (14086 atoms, 921 residues and 78390 AOs), where a total of 4096 vector processors (VPUs) of the Earth Simulator (ES) were utilized to complete the job within an hour. Later, the calculation with the extended 6-31G* basis set (121314 AOs) was carried out on cluster computers, and some specific residues associated with probable mutations

were successfully identified through the IFIE (inter-fragment interaction energy) analysis, thus providing a method to predict the forthcoming mutations in HA [3].

Although the second-order MP2 calculations have become feasible even for large proteins in conjunction with the FMO scheme, there has been a potential demand for correlated methods better than MP2. The third-order MP (MP3) theory can be a straightforward option by the perturbative inclusion of electron pair-pair interactions. Here, we employ an MPI-parallelized integral-direct implementation of FMO-MP3 scheme in ABINIT-MPX software. The OpenMP shared-memory parallelization is also introduced for the intra-fragment calculations of monomers and dimers at the lower level processing. The Earth Simulator, which was renewed in 2009 as ES2, is used as a massively parallel-vector computational platform, in which some technical points for the vectorization is addressed [4]. Then, we resort to a better calculation scheme, referred to as MP2.5 method, which utilizes a half-and-half mixture of the MP2 and MP3 energies [5].

2. Results

In the present study, we performed [4] the FMO-MP2 and FMO-MP3 calculations with the 6-31G or 6-31G* basis set for a complex consisting of HA trimer and two Fab fragments (2351 residues and 201276 AOs; PDB-ID: 1KEN) and a complex of NA and oseltamivir ligand (386 residues; PDB-ID: 2HU4) on the ES2. The modeling of the complex structures was performed with the aid of MOE software, in which the addition and structural optimization of hydrogen atoms were carried out. Table 1 compiles the timing data of benchmark calculations of HA and NA systems by using 64 nodes (total 512 VPUs) and 128 nodes (1024 VPUs) of ES2. The FMO-MP2 jobs were processed in 0.8 hours (48.3 minutes) for the HA monomer and in 4.3 hours (260.6 minutes) for the HA trimer with 128 nodes. Comparison of these timings illuminates the low scaling nature of the present FMO calculations. The acceleration from 64 to 128 nodes was slightly over 2, presumably due to the difference in background conditions. Nevertheless, a value close to 2 was expected because of an inherent parallelism of the FMO calculations. It is remarkable that the increase rate of computational cost by MP3 compared to MP2 is quite low. In

Table 1 Timing data for HA monomer, HA trimer and NA complex systems. The timing shown here is the turn-around job time in hours. Each node with eight VPUs was assigned to the intra-fragment calculations with OpenMP. The 64 node jobs were processed during the usual production run hours, whereas the 128 node jobs were performed under a dedicated usage with special permission. The 6-31G basis set was used throughout, except for the cases of HA monomer and NA with the asterisk (*) meaning the use of the 6-31G* basis set.

(System) Calculation level	Nodes	Time (hour)	Rel.[a]	Acc.[b]	TFLOPS
(HA monomer)					
FMO-MP2	64	1.7			0.97
FMO-MP3	64	2.7	1.6		2.27
FMO-MP4(SDQ)	64	4.7			4.78
FMO-MP2*	64	4.4			1.19
FMO-MP3*	64	8.7	2.0		3.02
FMO-MP2	128	0.8		2.1	2.06
FMO-MP3	128	1.3	1.6	2.1	4.67
(HA trimer)					
FMO-MP2	64	9.4			0.83
FMO-MP3	64	11.9	1.3		1.66
FMO-MP2	128	4.3		2.2	1.83
FMO-MP3	128	5.8	1.3	2.1	3.44
(NA)					
FMO-MP3	64	1.0			3.04
FMO-MP4(SDQ)	64	2.9			4.26
FMO-MP3*	64	4.4			3.09

[a]Cost factor of MP3 job relative to MP2 job.

[b]Acceleration due to the increase of VPUs from 512 to 1024.

particular, the FMO-MP3 calculation for the HA trimer, which might be the world's largest target system (36160 atoms) for correlated all-electron calculations to date, was completed in only 5.8 hours with 128 nodes. In the case of NA-oseltamivir, a favorable performance of FMO-MP3 calculation was obtained as well. As a whole, a realistic applicability of FMO-MP3 calculations to large-scale proteins has just been demonstrated with the ES2 system, while further improvements in the ABINIT-MPX code would still be required for better accuracy and efficiency. (In Table 1, the timing data for the most recent FMO-MP4(SDQ)/6-31G calculations are also compiled.)

Figure 1 shows the structure of HA trimer complexed with two Fab fragments. From the top view (b) of Fig. 1, it is observed that a hollow structure is formed by the bundled monomers (labeled with roman numbers and colors) and also that the Fab fragment is situated in contact with plural monomers. Figure 2 then illustrates the results of IFIEs calculated at the FMO-MP2.5/6-31G level for the complex consisting of the HA trimer and two Fab fragments. The interactions between the yellow domain and each colored residue are depicted in the figure, where the red and blue correspond to the attractive and repulsive interactions, respectively. On the basis of the evaluated interactions with the Fab fragment antibody, as shown in Fig. 2 (a), it would be possible [3] to enumerate those residues (marked in red) in HA that have a high probability of forthcoming mutations to escape from antibody pressure. This information about the probable mutations in HA would, in turn, facilitate the development of effective vaccines against influenza viruses. Further, the IFIE analysis between various domains in the complex would also be useful for the comprehensive understanding of the specific roles played by each domain in the complex.

For the probable mutations of amino acid residues in HA, the following two conditions should be satisfied [3]: That is, the mutant HA should preserve its viral function and also be able to escape the antibody pressure. The former condition is associated with the experimental work carried out by Nakajima et al. [6,7], in which they have extensively introduced single-point mutations in HA and measured the hemadsorption activity of the mutants to assess whether the mutated sites are allowed (positive) or prohibited (negative). The latter condition is associated with the present work in which attractive or repulsive interaction energies with the Fab dimer are evaluated in terms of the values of IFIE sum of the residues in the HA antigenic regions A and B (Fig. 2(b)). Our hypothesis [3] is that the residues satisfying these two conditions above (i.e., allowed site and attractive interaction) will be likely to mutate, which will be examined, in turn, through comparison with the historical facts concerning the actual mutations in HA.

We have evaluated the interaction energies between the

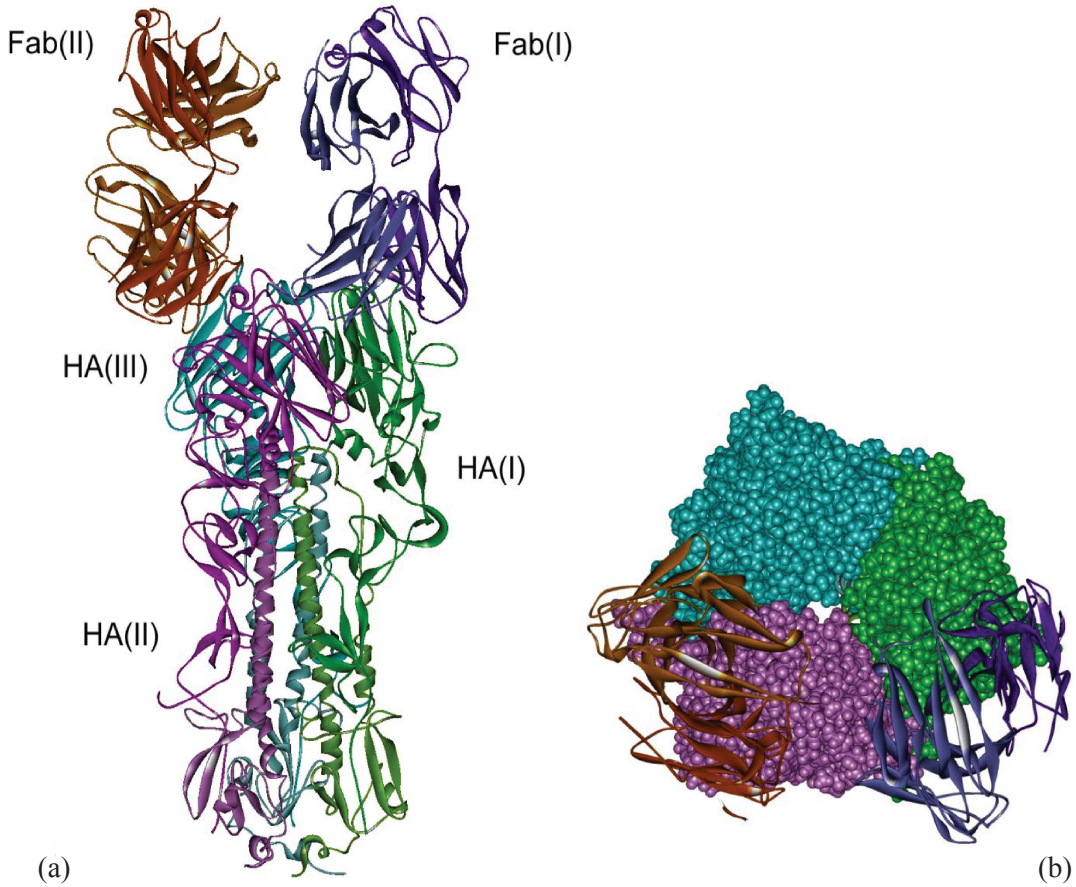

Fig. 1 Graphic representations of the influenza HA trimer with two Fab fragments: (a) side view, (b) top view. Each monomeric domain is identified with roman numbers and colors.

Fab dimer and all the amino acid residues in the HA antigenic regions A and B at the MP2.5/6-31G level. There are 21 residues of allowed and attractive sites which may be predicted to lead to mutations in our scheme. It is then observed that 17 residues of them have already been mutated. The other four residues may be expected to be mutated in future.

As is well known, the hydrophobic residues show smaller interaction energies with other residues than the charged and polar residues. In the present electron-correlated FMO calculations, we can quantitatively account for the dispersion interaction, which is typical of these weak interactions. There are 15 hydrophobic residues located at the allowed (positive) sites in the antigenic regions A and B of HA. Nine residues (121, 125, 142, 144, 146, 158, 163, 182 and 196) are under the antibody pressure, and seven of them have already been mutated. The residues PHE125 and VAL196 may be expected to be mutated in future. Although three residues (124, 135 and 143) show repulsive interactions with Fab dimer, they have been mutated. The substitution G135R enhances the attractive interaction with glycoprotein of host cell, and G135T enhances the attractive interaction with sialic acid. The remaining residues GLY124 and PRO143 interact with Fab monomers by 0-1 kcal/mol, which are very weak interactions.

Employing the HA trimer structure, we have obtained satisfactory results in fair agreement with the historical mutation data, as well as in the earlier study [3] in which the HA monomer structure was employed. Realistic trimer calculations for the mutation prediction based on the FMO method have thus been performed on the ES2 system. The details of the analysis will be reported elsewhere [8].

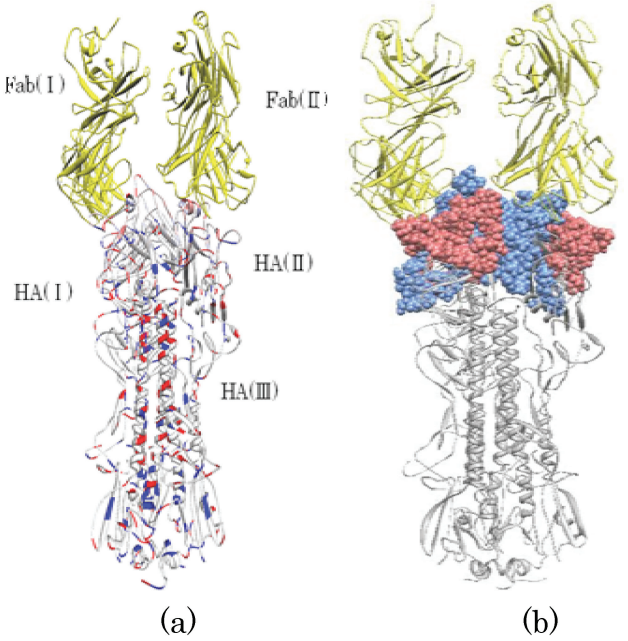

Fig. 2 (a) Visualization of IFIEs between HA trimer (I, II, III) and Fab dimer (I, II) calculated at the FMO-MP2.5/6-31G level. The color represents the sign and strength of the interactions between each residue in the HA trimer and the Fab dimer. For the Fab domain indicated in yellow, the red and blue fragments refer to stabilized and destabilized interactions, respectively, and the deepness of the hue indicates the strength of the interaction. (b) Visualization of antigenic regions A (pink) and B (light blue) by sphere representation. The illustration was generated with BioStation Viewer.

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References

[1] D.G. Fedorov and K. Kitaura, ed., *The Fragment Molecular Orbital Method: Practical Applications to Large Molecular Systems*, CRC Press, Boca Raton, 2009.

[2] Y. Mochizuki, K. Yamashita, T. Murase, T. Nakano, K. Fukuzawa, K. Takematsu, H. Watanabe, and S. Tanaka, Chem. Phys. Lett. 457, pp.396-403, 2008.

[3] K. Takematsu, K. Fukuzawa, K. Omagari, S. Nakajima, K. Nakajima, Y. Mochizuki, T. Nakano, H. Watanabe, and S. Tanaka, J. Phys. Chem. B 113, pp.4991-4994, 2009.

[4] Y. Mochizuki, K. Yamashita, K. Fukuzawa, K. Takematsu, H. Watanabe, N. Taguchi, Y. Okiyama, M. Tsuboi, T. Nakano, and S. Tanaka, Chem. Phys. Lett. 493, pp. 346-352, 2010.

[5] M. Pitonak, P. Neogrady, J. Cerny, S. Grimme, and P. Hobza, Chem. Phys. Chem. 10, pp. 282-289, 2009.

[6] K. Nakajima, E. Nobusawa, K. Tonegawa, and S. Nakajima, J. Virol. 77, pp. 10088-10098, 2003.

[7] K. Nakajima, E. Nobusawa, A. Nagy, and S. Nakajima, J. Virol. 79, pp. 6472-6477, 2005.

[8] A. Yoshioka, K. Fukuzawa, Y. Mochizuki, K. Yamashita, T. Nakano, Y. Okiyama, E. Nobusawa, K. Nakajima, and S. Tanaka, J. Mol. Graph. Model., in press, DOI: 10.1016/j.jmgm.2011.06.011.

フラグメント分子軌道法によるインフルエンザウイルスタンパク質の大規模電子状態計算と変異予測への応用

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フラグメント分子軌道（Fragment Molecular Orbital; FMO）法に基づき、インフルエンザウイルスの表面タンパク質に対する FMO-MP2 および FMO-MP3 計算を地球シミュレータ（ES2）を用いて行った。計算の対象としたのは、ヘマグルチニン（HA）三量体と Fab 抗体二量体の複合体（2351 残基、36160 原子）ならびにその単量体である。HA 単量体および三量体系に対して、電子相関を２次の Moeller-Plesset 摂動法で考慮した FMO-MP2/6-31G 計算は、ES2 の 128 ノード（1024 VPUs）を用いて、それぞれ 0.8 時間および 4.3 時間で完了した。このことは、本 FMO 計算がサイズ増加に対して良好なスケーリング関係を有することを示している。また、MP2 計算に対する３次の MP3 計算の相対的なコスト増加も極めて低く、例えば HA 三量体系に対する FMO-MP3/6-31G 計算は ES2 の 128 ノードを用いてわずか 5.8 時間で終了した。このように、これらの計算を通じて、大規模タンパク質系に対する FMO-MP3 計算が ES2 上で効率的に実行可能であることが示された。また、これらの FMO 計算の結果を用いて抗原－抗体系に含まれるフラグメント（アミノ酸）間の相互作用解析を網羅的に行い、HA タンパク質内のアミノ酸が抗体圧から逃れるためにどのように変異を起こすのか、その背景となるメカニズムを理論的に考察し、過去の変異の履歴をよく説明できることを確かめた。こういった分析手法はインフルエンザウイルスの将来の変異予測やワクチン開発等に役立てることができる。

キーワード: フラグメント分子軌道法, メラー・プレセット摂動法, インフルエンザウイルス, ヘマグルチニン