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REGULAR ARTICLE

Antigen–antibody interactions of influenza virus hemagglutinin revealed by the fragment molecular orbital calculation

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Abstract Effective interactions between amino acid residues in antigen–antibody complex of influenza virus hemagglutinin (HA) protein can be evaluated in terms of the inter-fragment interaction energy (IFIE) analysis with the fragment molecular orbital (FMO) method, in which each fragment contains the side chain of corresponding amino acid residue. We have carried out the FMO-MP2 (second-order Moeller–Plesset) calculation for the complex of HA antigen and Fab antibody of influenza virus H3N2 A/Aichi/2/68 and obtained the IFIE values between each amino acid residue in HA and the whole antibody as the sums over the residues contained in the latter. Combining this IFIE data with experimental data for hemadsorption activity of HA mutants, we succeeded in theoretically explaining the mutations in HA observed after the emergence of influenza virus H3N2 A/Aichi/2/68 in an earlier study, except for those of THR83. In the present study, we employ an alternative way of fragment division in the FMO calculation at the carbonyl C site of the peptide bond instead of the C_α site used in the previous work, which provides revised IFIE values consistent with all the historical mutation data in the antigenic region E of HA including the case of THR83 in the present prediction scheme for probable mutations in HA.

Keywords Influenza virus · Hemagglutinin · Antibody · Fragment molecular orbital · Inter-fragment interaction energy

Dedicated to Professor Akira Imamura on the occasion of his 77th birthday and published as part of the Imamura Festschrift Issue.

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Abbreviations

HA	Hemagglutinin
FMO	Fragment molecular orbital
IFIE	Inter-fragment interaction energy
MP2	Moeller–Plesset second-order perturbation

1 Introduction

Hemagglutinin (HA), a major antigenic protein of the influenza virus, is a homo-trimeric glycoprotein situated on the viral surface [1]. HA plays an important role in the early stage of infection such as the binding to the receptors (sialic acids) on the host cells and the trigger for the fusion between virus and endosome membranes. The receptor-binding site (RBS) is located on the membrane-distal globular domain of HA. The neutralizing antibody targeting the antigenic regions located around the RBS then prohibits the virus from binding to the receptors. Antigenic variants are generally selected during circulation of the viruses among human population [2]. In these variants (mutants), amino acid differences are observed in the antigenic regions compared to the original viruses. The structural analyses of HA-antibody complexes have provided the information about the amino acid residues on HA directly interacting with the antibody in the complexes. Amino acid changes (mutations) at these positions allow the virus to escape from the neutralizing antibody.

In an earlier study [3], we performed a quantum-chemical, electron-correlated second-order Moeller–Plesset (MP2) perturbation calculation [4] for the HA antigen–antibody system of influenza virus H3N2 A/Aichi/2/68 [5] with the fragment molecular orbital (FMO) method [6, 7], in which a large antigen–antibody biomolecular system was divided into a collection of many fragments corresponding to amino acid residues. On the basis of the calculated inter-fragment interaction energies (IFIEs) [8–14] representing the molecular interactions between the amino acid residues in the antigen–antibody complex, we identified those residues in the antigenic region E [15, 16] of HA protein that were significantly recognized by the Fab fragment of antibody [17, 18] with strongly attractive interactions. Combining these IFIE results with the data of hemadsorption experiments [19, 20] by which the mutation-prohibited sites were specified enabled us to explain most of those mutation sites actually observed (five of six residues) as a benchmark test, which would thus provide a promising method for predicting the HA residues that have a high probability of forthcoming mutation.

In spite of its success, only one residue site in HA has remained to be explained in the earlier study [3], whose mutation cannot be accounted for appropriately in terms of the proposed prediction scheme. That is the THR83 in the antigenic region E of HA, which shows a repulsive interaction with the Fab antibody in the FMO calculation, but has mutated three times [19, 20] after the emergence of the H3N2 influenza virus in 1968. In our prediction method, the amino acid residue that shows an attractive interaction with the antibody was supposed to have a high probability for mutation in order to escape from antibody recognition. One of the possible reasons for the disagreement with the observation can be ascribed to the fragment division method employed in the FMO calculation. We relied on the fragment division at the C_α site in the polypeptide chain according to the usual FMO recipe [6, 7, 21] for reducing the computational error in total energy, while this method of fragmentation may be less natural than the division at the peptide bond in light of biochemical function (see also the Sect. 4 below). Therefore, we here attempt an alternative method of fragment division for the IFIE calculation in the FMO method to overcome this difficulty. Concerning the accuracy of the calculated energies, the fragment division at the atoms other than the C_α site may cause additional energy errors in IFIEs by the order of less than 1 kcal/mol [6, 7, 21]. (Note that the IFIE values calculated with different fragmentations differ mainly due to the difference in the fragment units employed in the FMO calculations.)

The present article is organized as follows. In Sect. 2, the models and methods employed in the present study are illustrated. The calculated results obtained by the two kinds of the fragmentations are shown and compared in Sect. 3. We thus find in Sect. 4 that the IFIEs calculated through the fragmentation at the peptide bond give a description for the antigen–antibody interactions, which is more consistent with the main stream amino acid changes observed in the antigenic region E of HA. Section 5 concludes with a summary.

2 Models and methods

We employ an HA-Fab antigen–antibody system of H3N2 A/Aichi/2/68 influenza virus (PDB ID: 1EO8) [5] in the present analysis. Before performing the FMO-IFIE calculations, we added the missing hydrogen atoms in the complex and optimized their positions with the aid of MMFF94x force field [22] on the MOE (Molecular Operating Environment, Chemical Computing Group Inc.) software. Then, the FMO-MP2/6-31G* [3, 4] and the corresponding classical force-field (Amber ff99 [23]) calculations have been carried out for the antigen–antibody complex with 921 residues and 14,086 atoms, in which the monomer structure of HA [5] is employed. In contrast to

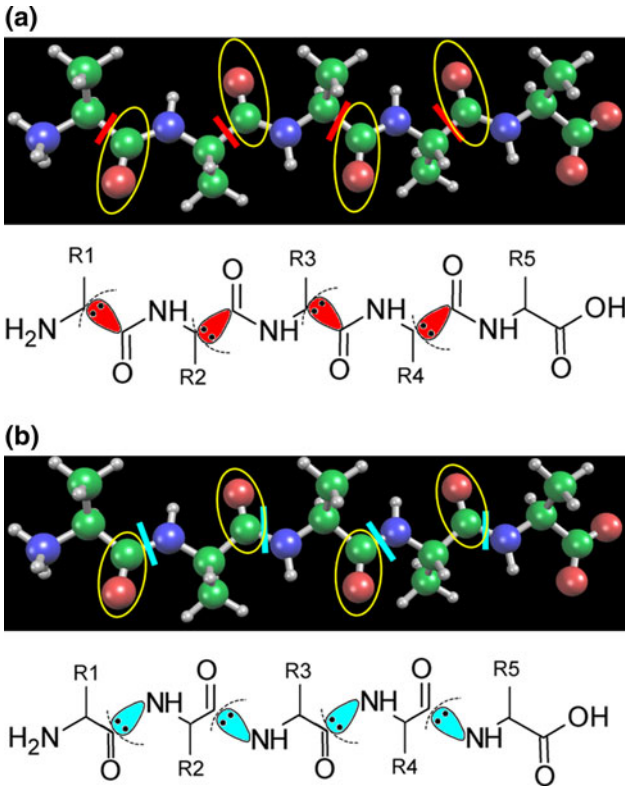

Fig. 1 Two methods of fragment division for the polypeptide chain. **a** The fragment division at the C_α site employed in the usual FMO calculations. **b** The fragment division at the carbonyl C site of the peptide bond employed in the present study. In the *lower panels*, the *dotted line* shows the fragmentation border, where chemical bond (orbital marked in *red* or *blue*) and electrons (represented by *dots*) are partitioned according the conventional FMO recipe [6, 7, 21]

the earlier study, where the fragmentation was performed at the C_α site of the C–C bond according to the usual FMO prescription [6, 7, 21], the fragmentation is performed at the carbonyl C site of the peptide (C–N) bond in the present analysis. The comparison between these two methods of the fragmentation is illustrated in Fig. 1.

We obtained the IFIEs [8–14] on the bases of these force-field and FMO (two-body FMO expansion [21] in this study) calculations and summed the values of IFIEs over the residues contained in the antibody. It is noted here that the IFIE is equivalent to the pair interaction energy (PIE) employed in other FMO studies [21]. These IFIE sums for each antigenic region A–E demonstrate that the amino acid residues in the vicinity of the antigenic region E are significantly recognized by the antibody through strongly attractive interactions, indicating that these residues are highly responsible for the binding affinity between the HA antigen and the Fab fragment of antibody. We therefore focus on 24 residues in the vicinity of the antigenic region E (residue numbers 62–85) in the present analysis.

It is noted, in passing, that we employ in this study an energetically optimized structure of complex on the basis of the PDB (Protein Data Bank) registered structure as a representative snapshot. Structural fluctuations of protein complex in environmental solvent are thus neglected in the present analysis, which may be justified by the consideration that the optimized structure should be observed dominantly by the energetical reason. It is also supposed that the screening effect due to solvent [24] would not change the relative order or the sign of IFIEs. In this regard, the MP2 electron-correlation method [3, 4, 8–14] is employed in the FMO calculation in order to appropriately describe the weak dispersion interactions in addition to other quantum-mechanical effects such as electronic polarization and charge transfer.

3 Calculated results

First, we show the result for the IFIEs between each residue in HA and the Fab fragment of antibody obtained by the classical force-field calculation, in which the fragmentation is performed at the carbonyl C site of the peptide bond. Figure 2a–c demonstrate the calculated results for charged, hydrophobic and polar residues in the antigenic region E, respectively, in comparison with the earlier FMO results to which the fragmentation at the C_α site was applied. As seen in the figures, the qualitative tendency of the interactions is well correlated in both calculations on the whole, while the classical force-field calculation seems to underestimate the contribution associated with the attractive dispersion interaction; the differences for the charged residues may be partially ascribed to the neglects of electronic polarization and charge transfer in the classical calculation. A significant, qualitative difference between the two calculations is then observed for those residues such as GLN80 and THR83, which may be attributed to the difference in the ways of fragment division as mentioned earlier. In particular, the latter residue is important in the present context of mutation prediction. If we would employ the IFIE value between THR83 and antibody obtained through the force-field calculation, we can assign this residue as a probable candidate for forthcoming mutation because the mutation at the THR83 site could diminish the currently attractive interaction with the antibody, which would be favorable for the escape from the antibody pressure. This is then consistent with the observation [19, 20] that the THR83 site has undergone mutations in the actual influenza viruses.

We have next carried out the FMO-MP2/6-31G* calculation for the identical antigen–antibody structure. In this calculation, we have modified the method of fragmentation from that employed in the earlier work, so that five residues including THR83 are separated at the carbonyl C site of the

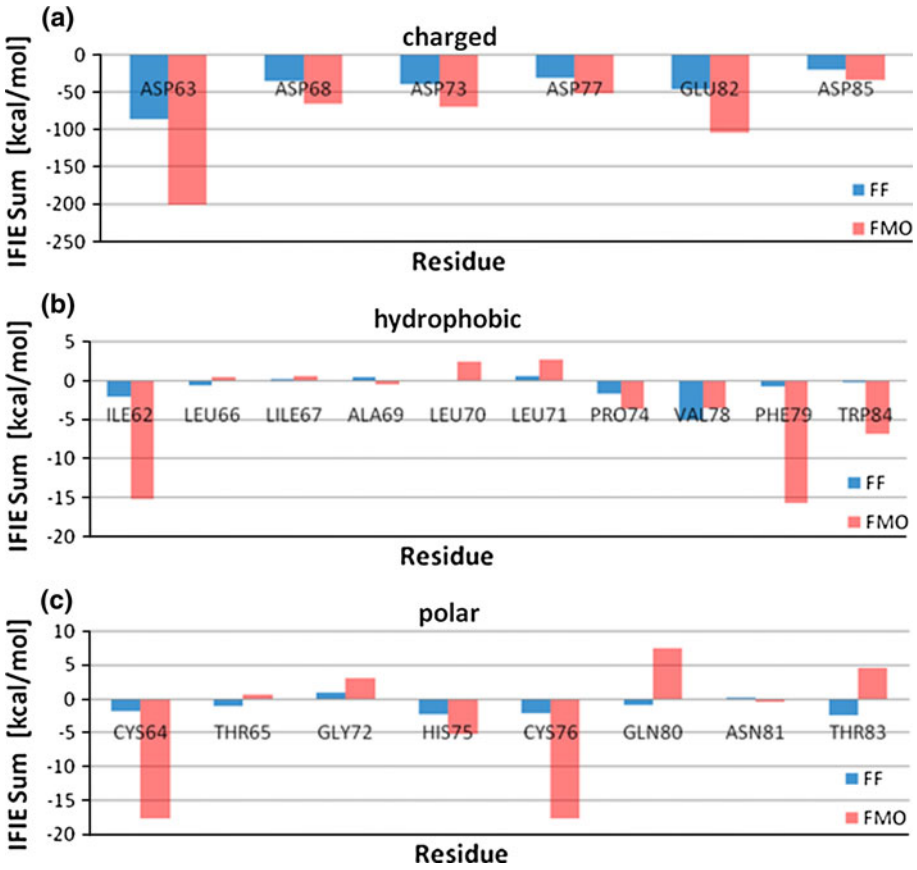

Fig. 2 IFIE values between the whole Fab antibody and each amino acid residue in HA. The *blue* and *red bars* represent the results obtained by the classical force-field (FF) calculation with the present fragmentation of Fig. 1b and by the FMO-MP2/6-31G* calculation with the usual (previous) FMO fragmentation of Fig. 1a, respectively. **a** Charged residues. **b** Hydrophobic residues. **c** Polar residues

peptide bond instead of the C_α site. Table 1 shows the IFIE values between each residue fragment and the antibody, which are compared between the previous and present ways of FMO fragmentation. Most interesting in this table is the IFIE values for THR83 and TRP84. The IFIE for THR83 changes from 4.52 kcal/mol in the previous scheme to −4.93 kcal/mol in the present scheme, which is in line with the result observed in the classical force-field calculation above; on the other hand, that for TRP84 changes from −6.85 to 1.25 kcal/mol. These results can be accounted for as consequences of the modification of the fragmentation, as will be addressed in the following section, and provide a consequence consistent with the observation [19, 20] for mutations of HA in the H3N2 influenza virus.

4 Discussion

As addressed in Sect. 2, the fragment assignment of the carbonyl group C=O in the main chain of polypeptide is different between the two types of fragmentations shown in Fig. 1; the fragmentation was performed at the C_α site in the usual FMO scheme, while it is performed at the carbon site of the carbonyl group C=O constituting the peptide bond in the scheme proposed in the present study. When we consider the IFIE between THR83 in HA and ARG98 in Fab antibody, this difference causes a significant consequence. Figure 3 illustrates the configuration of THR83 and ARG98 in the antigen–antibody complex. The carbonyl group next to the side chain of THR83 has an attractive interaction with ARG98. In the case of original FMO fragmentation, this carbonyl group belongs to the fragment containing the side chain of TRP84, then bringing about an attractive IFIE between the TRP84 fragment and the antibody (see −6.85 kcal/mol in Table 1). In the present fragmentation, on the other hand, this carbonyl group belongs to the fragment containing the side chain of THR83. Thus, the IFIE value between the THR83 fragment and the antibody becomes negative (−4.93 kcal/mol) in the present FMO calculation, as seen in Table 1, providing a calculated result consistent with the mutation data in terms of the present prediction scheme.

It is noted that the mutations at all six residue sites, which have been observed in the antigenic region E of HA of H3N2 A/Aichi/2/68, can thus be accounted for by the prediction method based on the present fragmentation scheme. This example would suggest that the fragmentation at the carbonyl site of the peptide bond may be a more natural way of fragment division in FMO calculation in

Table 1 IFIE values (in units of kcal/mol) between the Fab antibody and amino acid residues in the vicinity of THR83

Fragment	IFIE sum (kcal/mol)	Number of atoms
(a) Previous		
GLN80	7.47	17
ASN81	−0.45	14
GLU82	−102.51	15
THR83	4.52	14
TRP84	−6.85	24
ASP85	−34.93	12
LEU86	2.21	19
(b) Present		
GLN80	7.43	17
ASN81	0.63	16
GLU82	−101.98	15
THR83	−4.93	14
TRP84	1.25	24
ASP85	−37.41	10
LEU86	2.23	19

(a) The results obtained by FMO-MP2/6-31G* calculation employing the usual fragment division at the C_α site of the C–C bond shown in Fig. 1a. (b) The results obtained by FMO-MP2/6-31G* calculation employing the present fragment division at the carbonyl C site of the peptide (C–N) bond shown in Fig. 1b. The numbers of atoms contained in each fragment corresponding to amino acid residue (side chain) are also shown

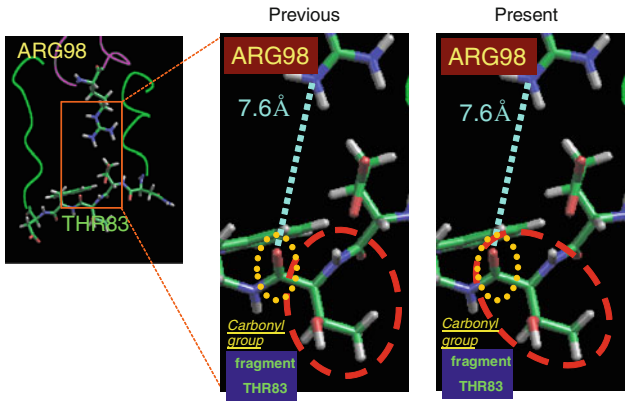

Fig. 3 Configuration of THR83 in HA antigen and ARG98 in Fab antibody. The side chain of THR83 and the carbonyl group interacting with ARG98 are *circled* by the *dashed red line* and the *dotted yellow line*, respectively. These two parts are contained in an identical fragment (THR83) only in the present fragmentation scheme

order to discuss biochemical consequences of mutations, while the original method of fragment division was employed to reduce the computational errors due to the fragmentation as much as possible in the FMO calculations [6, 7, 21]. In this connection, we suppose that the change of the side chain of amino acid residue would modify the local structure of neighboring region of the main chain as well, thus leading to the change in the interaction associated with the main chain. Thus, the effects of the mutations are indirect when the interactions associated with the main chain are important.

For more realistic analysis, we may employ the trimer structure [1] of HA antigen complexed with the Fab dimer antibody. The FMO calculations for this HA trimer system has already been performed [25] at the MP2 and MP3 levels. The prediction of mutations using this FMO-IFIE result and its comparison with that by HA monomer would be interesting, which will be reported elsewhere [26]. It is also remarked that the effect of fluctuating protein structures in aqueous solution on the antigen–antibody interaction [27], which is beyond the scope of the present study, is an issue to be investigated in the future.

5 Conclusion

Our prediction scheme for probable mutations in HA protein of influenza virus depends on the evaluation of interactions between amino acid residues in HA and associated antibody. If the interaction is attractive, the corresponding residue would have a higher probability of forthcoming mutation in order to mitigate the molecular recognition by the antibody. These interaction energies could be evaluated in terms of the IFIEs obtained through the FMO calculation for the antigen–antibody complex. Then, the method of the fragment division employed in the FMO calculation would be important in order to appropriately assign the local interactions associated with pertinent groups in amino acid residues to each fragment. It is noted that these fragmentations are somewhat empirical and lead to the arbitrariness in the IFIE values. In our previous analysis [3] on an HA antigen–antibody system, we relied on the conventional fragmentation scheme in the FMO calculation and found that the prediction scheme worked well for explaining five of the six past mutations in the HA of H3N2 A/Aichi/2/68 influenza virus, but leaving the mutations of THR83 unexplained. In the present study, on the other hand, we have performed a novel fragmentation at the carbonyl C site of the peptide bond, which is more consistent with the biochemical importance of the peptide bond moiety in protein structures. While this fragmentation may bring about increased errors of calculated energies in the FMO approximation, we have improved the agreement between the theoretical prediction and the past mutations observed in the antigenic region E of HA of H3N2 A/Aichi/2/68 influenza virus. This finding may thus suggest various options [28] for us to choose the method of fragmentation employed in the FMO calculation according to the purpose of analysis.

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