New Fragmentation of Fragment Molecular Orbital Method Applicable to Fragment Based Drug Design

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1 Introduction

Estrogen receptor (ER), which is a ligand-dependent transcription factor, is an important drug target of breast cancer. Inter-fragment interaction energy (IFIE) analysis based on the ab initio fragment molecular orbital (FMO) method has revealed ligand binding mechanism of the ER [1, 2]. The IFIE is one of the advantages of FMO method and can represent detailed interactions at the amino acid residue and the ligand levels. On the other hand, the IFIE analysis for each ligand binding site at the functional unit (e.g., by fragmentation of ligand) will be valuable to Fragment Based Drug Design (FBDD). However, a ligand fragmentation in the two-body FMO (FMO2) method has not kept appropriate accuracy to discuss chemical reactions. Recently, the four-body corrected fragment molecular orbital (FMO4) method [3, 4] was developed and applied with high accuracy to the subdivision into main and side chain fragments, which are smaller than conventional main chain fragment. Following this, we attempt to perform the FMO4 calculation by using the fragmentation of ligand distinguishing each functional group along with the fragmentation of protein divided into main and side chains. In this study, the IFIE of complex between ER and 17β-estradiol (EST) was analysed at the FMO4-MP2/6-31G level with new fragmentation in FBDD context.

2 Method

2.1 FMO4 method

A brief description of the ab initio FMO4 method [3] is given here. In the FMO4 method, a molecule or a molecular cluster is divided into small fragments, and the MO calculations on each fragment monomer, dimer, trimer, and tetramer are performed to obtain the properties of the whole system. The many-body effects are considered through the environmental electrostatic potentials. The total energy of FMO4 calculation was given by

$$E^{\mathrm{FMO4}} = \sum_{I} E'_I + \sum_{I>J} \Delta\tilde{E}_{IJ} + \sum_{I>J>K} \Delta\tilde{E}_{IJK} + \sum_{I>J>K>L} \Delta\tilde{E}_{IJKL}$$

where E'_I is monomer energy without the environmental electrostatic potential, $\Delta\tilde{E}_{IJ}$, $\Delta\tilde{E}_{IJK}$ and $\Delta\tilde{E}_{IJKL}$ are two-body, there-body and four-body inter-fragment interaction energies and *I*, *J*, *K*, and *L* are fragment indices. Intermolecular interaction between ER and EST was calculated by a pharmacophore model, which consists of EST, a water molecule, and 50 residues of ER around EST, at the FMO4-MP2/6-31G level with the ABINIT-MP program [4].

2.2 New Fragmentation applicable to FBDD

Fig. 1 shows fragmentation of protein: (a) Main and (b) Main/Side chains. Each amino acid residue of ER was divided into main and side chain fragments in order to discriminate their contributions. In Fig. 2, the ligand was split into two fragments, EST(1) and EST(2) including "A ring" and "D ring" of steroidal EST, respectively, along with two ligand binding sites of EST, as shown in green and orange areas.

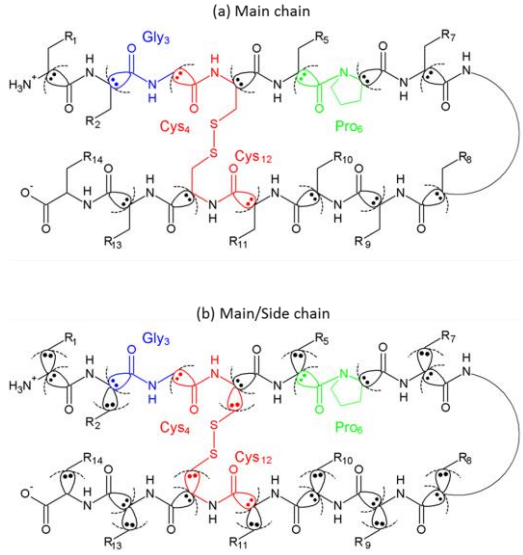

Fig. 1. Fragmentation of protein (a) Main chain and (b) Main/Side chain

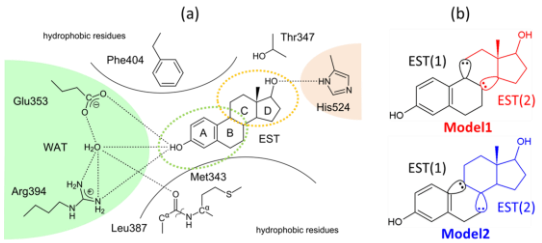

Fig. 2. (a) Ligand binding network of ER-EST complex and (b) Fragmentation of ligand

3 Results

3.1 Accuracy of total energy of FMO2, FMO3, and FMO4 calculations with new fragmentation

Table 1 lists the total energy errors of FMO2, FMO3, and FMO4 calculations with the fragmentations of Main and Main/Side chains for protein and that of Model1 and 2 for ligand, where the reference energies (in units of hartree) by the FMO calculations with the conventional Main chain fragmentation are included. It is clear that the accuracies of FMO2 calculations are not chemically sufficient at all levels. The accuracy of FMO4-HF/STO3-G calculations with the Main/Side chain fragmentations is equivalent to that of FMO3 calculation with the conventional Main chain fragmentation. These tendencies of energy errors more significantly appear at HF and MP2 levels with 6-31G basis. Here, the total energies of Model1 and Model2 are not very different. Thus, it is reasonable to employ FMO4 calculation instead of FMO2/3 calculation in order to perform the Main/Side chain and the ligand fragmentations. Next, we show intermolecular interactions between ER and each functional group of EST by using the FMO4-IFIE analysis with the Main/Side chain and the ligand (Model1) fragmentations.

Table 1. Total energy of FMO2, FMO3 and FMO4 calculations

Fragmentation		[c-e]Difference of total energy (hartree)		
Protein	Ligand	FMO2	FMO3	FMO4
HF/STO-3G ([c]Rerefence of total energy: -22578.9424 hartree)				
[a]Main chain	[c] No	0.0167	-0.0010	-
[b]Main/Side chain	[c] No	10.1236	-0.0068	0.0024
	[c] Model1	10.1247	-0.0066	0.0027
	[c] Model2	10.1256	-0.0065	0.0028
HF/6-31G ([d]Rerefence of total energy: -22855.8634 hartree)				
[a]Main chain	[d] No	-0.0227	0.0032	-
[b]Main/Side chain	[d] No	10.0978	-0.0423	-0.0028
	[d] Model1	10.0988	-0.0421	-0.0027
	[d] Model2	10.1007	-0.0420	-0.0028
MP2/6-31G ([e]Rerefence of total energy: -22898.2895 hartree)				
[a]Main chain	[e] No	-0.0026	-0.0001	-
[b]Main/Side chain	[e] No	10.3742	-0.0479	-0.0015
	[e] Model1	10.3776	-0.0461	0.0002
	[e] Model2	10.3797	-0.0462	-0.0001

[a] Divided at Cα atoms. [b] Divided at Cα and Cβ atoms.
[c-e] Compared with the FMO4 calculation at each level with the conventional Main chain fragmentation.

3.2 Inter fragment Interaction between ER and EST

Fig. 3 shows the FMO4-IFIEs of EST(1) and EST(2) with surrounding residues. Subscripts of residue name, "M" and "S", are show the Main and Side chain fragments, respectively (e.g. $Thr347_M$ and $Glu353_S$). Either EST(1) or EST(2) are coloured yellow, and the attractive and the repulsive interactions are coloured red and blue, respectively. The ligand binding mechanism of ER was elucidated in detail by analyzing the interactions between each functional group of EST and either main or side chain of each amino acid residue. EST(1) interacts with the main chain of Thr347, the side chains of Glu353, Arg394, and Phe404. In contrast, EST(2) interacts mainly with the side chain of His524. Thus, the intermolecular interactions between ER and EST at each ligand binding site are specified by the FMO4-IFIE analysis with the new fragmentation.

4 Conclusion

The FMO4 calculation with the new fragmentations of protein and ligand was performed for ER-EST complex in order to quantitatively reveal the key substructures of amino acid residue (main and side chains) and functional groups of ligand (EST(1) and EST(2)) with important roles for the ligand binding mechanism. In order to subdivide fragments into smaller ones, we confirmed that the total energy of FMO4 calculation is more appropriate than that of FMO2/3 calculation. The FMO4-IFIEs between ER and EST revealed that the functional group including "A ring" of EST (EST(1)) interacts with the main chain of Thr347 and the side chains of Glu353, Arg349, and Phe404; EST(2) including "D ring" of EST interacts mainly with the side chain of His524. Thus, the FMO4 calculation is available for analysis of intermolecular interaction for each ligand binding site at the functional unit. In addition, the FMO4-IFIE analysis with the new fragmentation and CHPI analysis [5] will be useful to specify a weak interaction such as CH/π interaction. Altogether, the FMO4-IFIE analysis by using the fragmentation of ligand may be helpful in drug discovery for FBDD.

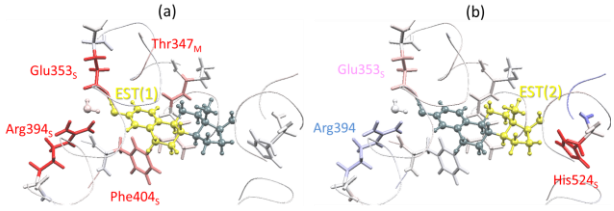

Fig. 3. FMO4-IFIEs at MP2/6-31G level of EST(1) and EST(2)

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