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Study on the mechanism of the West Nile Virus NS3 protease inhibitor using the Divide and Conquer method

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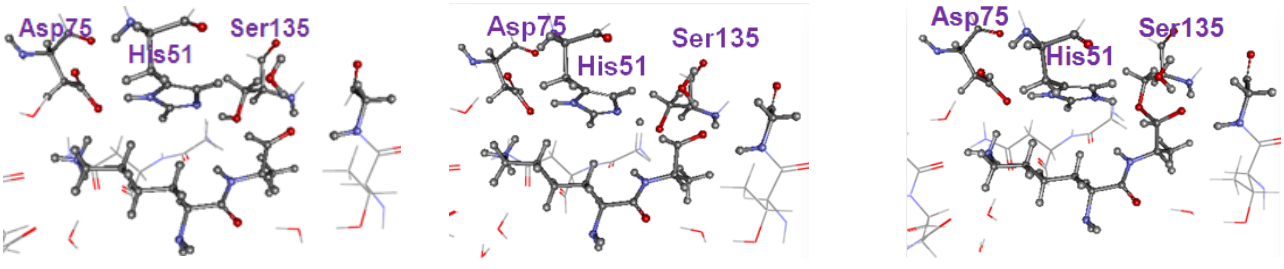
○ Patchreenart Saparpakorn [1], Masato Kobayashi[1, 2], Hiromi Nakai[1, 3, 4]

Introduction

West Nile Virus (WNV) is a member of the *Flavivirus* genus and is transmitted through mosquitoes. Currently, there is no effective vaccine and drug against WNV for human. In viral replication, WNV NS2B-NS3 protease plays an important role in the step for posttranslational cleavage of the substrate. 2-naphthoyl-Lys-Lys-Arg-H (Naph-KKR-H) is an effective substrate-based tripeptide inhibitor which was found to interact with NS2B-NS3 protease's active site in the similar way as substrate. Crystal structure complex between WNV NS2B-NS3 protease and Naph-KKR-H, pdb code 3e90, reveals two forms of Naph-KKR-H's binding in the active site [1]. In addition, the C-terminal aldehyde of Naph-KKR-H forms the covalent bond to a catalytic amino acid, Ser135. This form is called as tetrahedral intermediate (TI) form. Based on the conformation taken from the crystal structure, some water molecules in the binding pocket were also found to play an important role to stabilize or form the water-mediated interaction between the inhibitor and some amino acids. However, the mechanism of this inhibitor is proposed as an induced-fit mechanism [1]. In this study, one form of the inhibitor's binding is selected to study its mechanism by using divide and conquer (DC) method.

Methodology

The models consisting of catalytic triad (His51, Asp75, and Ser135), six surrounding amino acids, five water molecules, and a part of Naph-KKR-H forming the covalent bond are partially optimized and varied along the reaction coordinate of covalent bond forming using HF/3-21G* method. The Synchronous Transit-Guided Quasi-Newton (STQN) method is used to locate the transition state (TS). TI, TS and starting binding (Michaelis complex, MC) forms, as shown in Fig. 1, are refined without the restraint of reaction coordinate and checked with the frequency calculations. All models are performed single point energy calculation at DC-B3LYP, DC-MP2 and DC-SCS-MP2 methods [2,3] with 6-31G** basis set in both gas phase and CPCM model (water and $\varepsilon = 4$ used as solvent). Furthermore, effective fragment potential (EFP) method is also studied by treating with five water molecules.



MC **TS** **TI** (crystal structure's form)

Fig. 1 Structures of Michaelis complex (MC), transition state (TS), and tetrahedral intermediate (TI)

Results and Discussion

From the partial optimization, two conformation of His51 are found. Conf.1 is similar to the conformation in crystal structure and Conf.2 reveals the flip of imidazole ring. For the energy profile according to the reaction coordinate, local minima of TI forms cannot be found with the gas phase calculations. In the CPCM models, solvent models as water and $\varepsilon = 4$ give the similar trend of the results. Relative total energies of Conf.1 are about 2-4 and 1-2 kcal/mol lower than those of Conf.2 for DC-B3LYP and DC-SCS-MP2 methods, respectively. For DC-MP2 method, relative total energies of Conf.1 are close to and lower about 1 kcal/mol than those of Conf.2, but relative total energy of Conf.1 at TS is lower than that of Conf.2 at a point before TS. According to the energy profiles of three methods, we conclude that pathway of Conf.1, which is no flip of His51, is more preferable. Energy profile of DC-SCS-MP2 method is shown in Fig. 2. From the results, solvation models using the solvent with dielectric constant of water and $\varepsilon = 4$ show the similar energy barrier. Energy barriers obtained from DC-MP2 method are lower than those obtained from DC-B3LYP and DC-SCS-MP2, respectively. For the reaction energy, solvation models with dielectric constant of $\varepsilon = 4$ give the reaction energies higher than the models with dielectric constant of water about 2 kcal/mol. The order of the reaction energy in each method is as follows; DC-MP2 < DC-B3LYP ≈ DC-SCS-MP2. In the system that water molecules are treated as EFP (Model 2), the energy barriers are close to the results of the models including the water molecules in ab initio calculations (Model 1) but the reaction energies are about 3 kcal/mol higher than the another one. In case of the interaction energies of MC forms, Conf.1 and Conf.2 reveal the similar interaction energies. Models with the $\varepsilon = 4$ give the larger interaction energy than those with the water solvent. EFP shows less effect for the interaction energy. This study reveals the mechanism of Naph-KKR-H's binding to WNV NS2B-NS3 protease with the use of DC methods combined with the CPCM model.

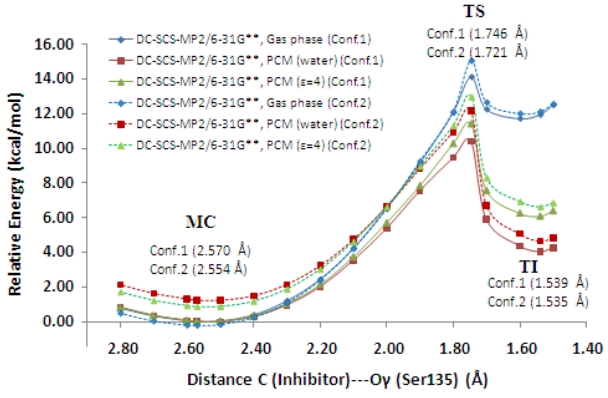


Fig 2. Energy profile of DC-SCS-MP2 method.

The energy barriers obtained from this study are about 7-13 kcal/mol. The reaction energies are presented as endothermic reaction. Dielectric constant and EFP only affect with the TI models, as shown in the reaction energies, about 3-4 kcal/mol.

Table 1. Energy barrier, reaction energy, and interaction energy in kcal/mol

Method	Solvent	Energy barrier (kcal/mol)		Reaction energy (kcal/mol)		Interaction energy of MC form (kcal/mol)			
		Model1	Model2	Model1	Model2	Model1*		Model2	
						Conf1	Conf2	Conf1	Conf2
DC-B3LYP/6-31G**	water	8.99	10.28	4.18	7.10	-54.92	-53.52	-53.59	-51.97
	ε=4	9.99	11.20	6.24	9.07	-82.48	-81.46	-81.45	-80.25
DC-MP2/6-31G**	water	6.85	8.81	1.25	5.05	-73.36	-72.28	-71.56	-70.12
	ε=4	7.85	9.73	3.24	7.01	-101.03	-100.32	-99.27	-98.23
DC-SCS-MP2/6-31G**	water	10.41	11.90	4.04	7.17	-63.97	-62.83	-64.40	-62.94
	ε=4	11.42	12.85	6.08	9.17	-91.75	-90.98	-92.20	-91.14

* 5 water molecules are included in ab initio calculations (Model 1) and are treated as effective fragment potential (Model 2)

References

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