Theoretical studies on association/dissociation process of protein complex related to protein-protein and ligand-receptor interactions

Abstract

Theoretical studies on association/dissociation process of protein complex related to protein-protein and ligand-receptor interactions

タンパク質ータンパク質相互作用及びリガンドー受容体相互作用に関連するタンパク質複合体の会合解離過程に関する理論的研究

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Abstract

The free energy profile as a function of the distance between the center of mass position of small ligand, i.e., 4-carboxyethyl benzene-sulfonamide ethyl ester, and CA I enzyme has been investigated by using all-atom MD simulation combined with thermodynamics method. We find that the electrostatic energy is the predominant impact for determining the binding free energy of the CA-I/ligand complex. From our results, the binding free energy of ligand/CA I complex from the reference state becomes -53.11± 14.17 kcal/mol and the free energy reaches the minimum at r_{cm} 8.5 Å. This result is consistent with that of the equilibrium MD, in which the average value is r_{cm} 8.58 Å. In the case of the interaction between proteins, we have investigated the binding energy of plastocianin (Pc) and cytochrome f (Cyt$f$) complex by using all-atom MD simulation. The force field parameters in the active site of plastocianin and cytochrome f are calculated by quantum chemical calculation. From our calculation, the binding free energy of Pc-Cyt$f$ complex becomes -32.34± 1.82 kcal/mol. Additionally, due to interest on association/dissociation process of plastocianin and cytochrome f complex, we have performed parallel cascasde selection molecular dynamics (PsCS-MD) on those protein complex and have been presented in this paper. Also, the free energy landscape (FEL) is estimated by using multiple independent umbrella sampling (MIUS) around conformational sampling at the constraint distances. From our calculation, we obtain the flat energy at the distances 37 to 44 Å. The selected structures around the flat energy are used to analyze the conformation changes of the Pc-Cyt$f$ complex. The stability of RA-VII in complex with 60S ribosome is also presented. We find twenty models from docking simulation. From our results obtaining by docking, RA-VII in model 1 becomes a promising drug for inhibiting 60S ribosome.
Keywords: ligand-receptor complex, Pc-Cyt$f$ complex, binding free energy, association/dissociation process, free energy landscape, conformational analysis.

1 Introduction

1.1 Research Background and Motivation

In organisms, carbonic anhydrase (CA) isozymes can be found from archaea, prokaryotes, and eukaryotes [1]. In human, some diseases, i.e., glaucoma, diabetes, cancer, epilepsy, and etc., are connected almost every CAs family. CAs enzymes involve the biological process in celss such as catalyzes of the hydration of carbon dioxide to bicarbonate which is essential to regulate the pH levels in cells, biosynthetic reaction and electrolyte secretion in several tissues [1–3]. Several ligand molecules as inhibitors to inhibit CAs activity are the critical target for the therapeutics against many diseases. Understanding the interaction between ligand and CAs in relation to the thermodynamics properties becomes important to understand the free energy change of the ligand molecules in the binding/dissociation process. Therefore, we perform all-atom molecular dynamics (MD) simulation combined with thermodynamic integration method to estimate the free energy profile for binding/dissociation process of ligand from CA I enzyme. Furthermore, the force field parameters of the zinc ion in the CA I active site is estimated by quantum chemical calculations. In this section, we discuss the stability of CA I-ligand complex related to some thermodynamic properties such as the binding free energy, the equilibrium state of the free energy surface and so on.

In the case of the interaction between proteins, we investigate the behavior of plastocianin with cytochrome f (Pc-Cyt$f$ complex) in chemical reaction is an unique system for study of interprotein electron transfer because the reduction and oxidation processes by the electron transfer are rapid between the complex of the soluble domain in Cyt$f$ and soluble Pc. In the electron transfer reaction, the interaction of Pc/Cyt$f$ complex has been experimentally investigated by some groups [4–7]. Crowley and coworkers have presented the structure of Pc/Cyt$f$ complex in respect to the hydrophobic interaction [4]. The contribution of the hydrophobic and the electrostatic interactions of those proteins have been discussed to find the possible structure of of the weak and short-lived complex [5, 6]. Also, the diffusion of plastocianin and interaction with cytochrome f in the environment of the thylakoid membrane have been discussed [8–11]. In our previous studies, the hydrophobic interaction arising water in Pc/Cyt$f$ complex has been carried out by coarse-grained (CG) model [11]. The concept of molecular crowding effects is presented to evaluate the decreasing of the hydrophobic interaction around the contacting area of the complex crowded with many hydrophobic residues. All-atom molecular dynamics simulations have been performed by estimating the binding free energy of Pc/Cyt$f$ complex before and after the electron reaction in relation to the association/dissociation process [12]. From our calculations, we obtain the total binding free energy of those reactions becomes 4.4 kcal/mol and the free energy obtained from all-atom MD simulation corresponding to the association/dissociation process becomes similar to that obtained by the coarse-grained model. However, the detailed analyses of the association/dissociation pathways have not been investigated. These pathways become crucial due to conformational changes of Pc/Cyt$f$ complex around the association/dissociation.

In the case of interaction between ligand and receptor, we also investigate the stability structure between RA-VII and 60S ribosome. RA-VII is one of a series of bicyclic hexapeptides which can be isolated from *Rubia cordifolia* L. and *R. akane* Nakai (Rubiaceae). The anti-tumor action of RA-VII is considered to be due to the inhibition of protein synthesis through binding to eukaryotic ribosome[13]. From the late 1980s to early 1990s, RA-VII underwent clinical trials as an anti-cancer agent in Japan [14]. Therefore, it is important to study the stability of the binding of RA-VII in the pocket of 60S subunit to develop an anti-cancer drug through inhibiting protein synthesis.

1.2 Research Objectives

To understand the association/dissociation process of protein complex related to the protein-protein and ligand-receptor interaction, we discuss the binding/dissociation process of ligand molecule by calculating the free energy profile to know the stability of CA I-ligand complex related to some thermodynamic properties such as the binding free energy, the equilibrium state of the free energy surface and so on. We also perform all-atom molecular dynamics simulation to calculate the free energy profile of plastocianin in binding with cytochrome f. Also, the conformational transition pathways of plastocianin and cytochrome f in respect to the association/dissociation process by PaCS-MD simulation developed by Harada and co-authors [15] is performed. This method has been used to find the possibility of the conformational transition pathway of folding process starting from the extended structure of chignolin protein to the native structure along simulation. The folding pathway obtained by PaCS-MD is good correspondence with the results presented in Ref. [16]. Therefore, we adopt this method to find the possibility of the association/dissociation pathway of Pc/Cyt$f$ along simulation. On the other hand, the binding free energy between CA I enzyme and the ligand molecule is investigated. Also, we investigate the possibility of the binding of RA-VII molecule into the 60S ribosome by using molecular docking simulation.

2 Computational Method

2.1 Free energy calculation

In order to obtain the binding free energy between ligand and receptor or for the protein complex, we calculate the complex by generating the free energy profile $\Delta G(r)$ as a function of the distance, r, between the center of mass of ligand and CA I enzyme as follows [19, 20]:

$$\begin{aligned}\Delta G(r) &= G(r) - G(r_0) \\ &= \int_{r0}^{r} (\frac{dG(r')}{dr'}) dr' \\ &= \int_{r0}^{r} < \frac{\partial U}{\partial r'} >_{r'} dr,'\end{aligned} \tag{2.1}$$

where U is the potential energy of the whole system, r_0 is a reference distance, $G(r)$ is the free energy of the whole system, and $< ... >_r'$ represents the isothermal-isobaric ensemble average, where the distance r_{cm} is constrained to r'. The free energy profile is evaluated by [20, 21] as follows :

$$\Delta G(r) = -\int_{r0}^{r} < F(r') >_{r'} dr'$$

$$F(r') = (\frac{m\mathrm{Cyt}}{m\mathrm{Cyt} + m\mathrm{Pc}} \boldsymbol{F}\mathrm{Pc} - \frac{m\mathrm{Pc}}{m\mathrm{Cyt} + m\mathrm{Pc}} \boldsymbol{F}\mathrm{Cyt}).\boldsymbol{n}, \tag{2.2}$$

where $F(r')$ is the mean force acting between the center of mass of the CA I enzyme and that of ligand; $\boldsymbol{F}$Pc and $\boldsymbol{F}$Cyt are forces acting on the center of mass of CA I enzyme and that of ligand, respectively; mCyt and mPc are the total masses of CA I enzyme and ligand, respectively; and $\boldsymbol{n}$ is the unit vector from the center of mass of CA I enzyme to that of the ligand molecule. Then, $\Delta G(r)$ is calculated as follows [17, 22]:

$$\Delta G(r) \cong -\sum_{i=1}^{Nw} \frac{1}{2}(< F(r') >_{r'=r_i} + < F(r') >_{r'=r_{i-1}})(r_i - r_{i-1}), \tag{2.3}$$

The free energy profile of the CA I enzyme/ligand complex as a function of the distance between the center of the CA I enzyme and that of ligand can be calculated using $< F(r') >$ from trajectories of MD simulations.

2.2 Simulation condition

All-atom molecular dynamics simulation is performed on CA I enzyme and ligand molecule. The TIP3P water model [23] with 9808 water molecules inserted in a 70 $\times$ 69 $\times$ 77 Å^3 periodic box then, 3 Cl^- ions are added to neutralize the whole system. The total number of atoms in the system is 33,425. The AMBER14 force field is used for the protein molecule [**?**], and general AMBER force field (GAFF) is applied to determine the force field parameters of ligand molecule. The electrostatic interactions are computed by using the Particle Mesh Ewald (PME) algorithm. The switching cutoff distance is 10 Å. The SHAKE algorithm is used to constrain the bond distance of the hydrogen atoms. A time step of 2 fs is applied in all simulations. The energy minimization of the system is carried out with the constraint on the position of the heavy atoms of CA I, ligand and Cl^- ion. Then, we perform the energy minimization without any constraint. The system is simulated on NVT-constant simulation for 60 ps where the temperature is gradually increased from 0 to 300 K. The temperature and pressure of the system are kept at 300 K and 1 atm by using the Langevin thermostat and isotropic position scaling algorithm, respectively. We equilibrate the system with the NPT ensemble for 50 ns without the harmonic positional constraint. After the whole system reaches the equilibrium state, the production run with a constraint on the distance between the centers of mass of CA I active site and the ligand is performed. The SHAKE method is used to constraint the distance. We prepared 19 distances with the increment of 0.5 Å: r= 6-15 Å. Each simulation is performed for 5 ns with NPT-constant MD simulation to obtain the mean force $< F(r') >$ from the MD trajectory. All MD simulations are carried out by Amber 16 packages [**?**]. The analysis of the trajectories of MD simulation is performed by CPPTRAJ tools [24].

2.3 Molecular Docking Simulation

Molecular docking simulation is performed by using AutoDock Vina packages to obtain the binding pocket for RA-VII in the 60S ribosome [25]. The docking simulation was performed by determining the grid box size to cover all the complex between RA-VII and 60S ribosome. The grid box size is set on 126 $\times$ 126 $\times$ 126 points with a grid spacing of 1.00 Å. The center of the grid box was placed at (x, y, z)=(8.079, -266.256, 88.683). The molecular structure file formats of RA-VII and 60S ribosome sre converted to PDBQT format. All of those docking parameters are prepared by using AutoDock Tools 1.5.6.

3 Results and Discussions

3.1 Binding free energy of ligand from CA I active site

To obtain the free energy profile as a function of r_{cm} of ligand/CA I complex, firstly, we estimate the mean force $< F(r') >$ for 19 distances. We find that interaction between CA I and ligand molecule almost vanishes at 15 Å because the mean force at this position becomes small at around 5.4 x 10^{-11} N. Thus, we decide that the distance of 15 Å becomes the reference state. Furthermore,

the results obtained by the mean force calculation are used to determine the free energy profile as a function of r_{cm}. From our results, the binding free energy of ligand/CA I complex from the reference state becomes -53.11± 14.17 kcal/mol and the free energy reaches the minimum at r_{cm} 8.5 Å. This result is consistent with that of the equilibrium MD, in which the average value is r_{cm} 8.58 Å. Although, the calculated binding free energy looks bigger with the value of -53.11 kcal/mol, for the case of the binding free energy of protein/ligand complex, the energy could be around -53.11 kcal/mol or more [17, 26, 27].

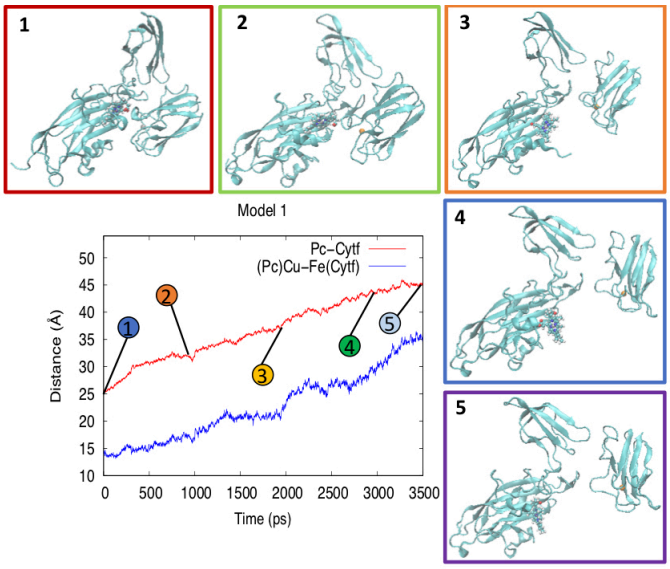
To obtain the binding free energy from the standard state, we investigate the binding free energy of the protein-ligand complex in water solvent by estimating the gas-phase interaction energy E_{MM}, solvation free energy E_{Solv}, and entropy $-T\Delta S$ of the complex. We obtain that the binding free energy for all CA I/ligand complex is good agreement with the experimental results. Also, we find that the electrostatic energies (ΔE_{ele}) are different with values of -119.25, -166.54, -129.35, -130.79 kcal/mol. We conclude that the electrostatic term influences for determining the different binding orientations.

3.2 Free energy profile of Pc-Cyt$f$ complex

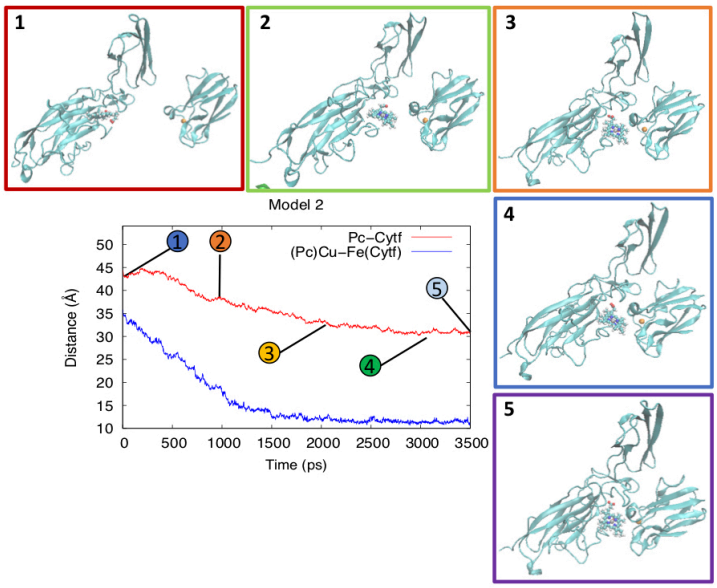
The binding free energy from the reference state is calculated by using thermodynamic integration method to evaluate the dissociation of plastocianin in binding with cytochrome f. In our method, we calculate the binding free energy from the reference state (R_{CM}= 45 Å) and not from the equilibrium binding constant. The binding free energy is estimated by using thermodynamic integration method to elucidate the dissociation process of the plastocianin in complex with cytochrome f along the reference distance. The binding free energy of the complex from the reference state become -32.34± 1.82 kcal/mol and the free energy reaches the minimum at the distance of 29 Å. This result is consistent with that of the equilibrium MD, in which the average value of the distance between the center of mass of plastocianin and cytochrome f for the last 2 ns is around 28.99 Å. Therefore, this letter may contribute to present an insight approach for estimation of the free energy profile as a function of the binding distance.

3.3 Association/dissociation pathways of plastocyanin and cytochrome f complex by PsCS-MD

Figure 1(a) in red line shows the distance between center of mass of Pc/Cyt$f$ complex along top trajectories corresponding to model 1 calculated by PaCS-MD simulation. The first cycle of PaCS-MD is started from the preliminary structure is shown in Figure 1(a) at the snapshot number 1. This snapshot structure is selected based on the furthest distance between proteins. As shown in Figure 1(a), the time simulation of 3500-ps MD corresponding to 35 cycles of 10 MIMD for 100-ps is sufficient to generate the dissociation pathway of plastocianin from the side of cytochrome f. This indicates that strong structural selection in each cycle is needed for fast dissociation of plastocianin outside from cytochrome f. To examine the detail of the association process of Pc/Cyt$f$ complex, we also perform PaCS-MD simulation with similar procedure calculation presented in the dissociation process. Model 2 is constructed by locating plastocianin far away from the side chain of cytochrome f. We define that proteins in complex do not have hydrophobic and acid interactions between their side chains. The distance between center of mass of Pc/Cyt$f$ complex along top trajectories is presented in Figure 1(a). From Figure 1(a), we show the association pathway of plastocianin into cytochrome f along simulations, and the reactive trajectories are obtained during 3500-ps MD corresponding to 35 cycles of 10 independent MD for 100-ps.



(a)



(b)

Figure 1: The red line represents the distance between center of mass position of Pc/Cyt$f$ complex and blue line represents the distance between metals of iron and cupper ions corresponding to models 1 and 2 to describe the dissociation pathway of plastocianin in complex with cytochrome f. (a) The dissociation pathway of plastocianin in complex with cytochrome f, (b) The association pathway of plastocianin in complex with cytochrome f.

3.4 Stability of RA-VII for Anti-cancer Agent by docking simulations

In order to obtain the information about the possible binding site of the 60S ribosomal subunit for the RA-VII molecule and the conformation of RA-VII in the complexes, molecular docking simulation has been performed by using AutoDock Vina software without including the effect of solvation. Twenty complexes are obtained by the simulations, and their binding affinities are provided in Table 1. Model 1 has the lowest binding affinity. Although the differences of the binding affinity among those twenty models are small, the conformational structure of RA-VII in each binding model is different. The conformation of RA-VII molecule is important for the anti-tumor activity [33]. From our simulations, we only select six models then focused on other physical properties by analyzing the conformational changes of RA-VII molecule and the binding site between the ligand molecule and the bases of the 60S ribosomal subunit in the complexes which may be related to stability of the binding of RA-VII into the 60S ribosome. Also, from our results by docking simulation, model 1 has the lowest binding affinity and may become a promising drug for anti-tumor.

Table 1: Binding models obtained by docking simulation with AutoDock Vina.

Model	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
Binding affinity (kcal/mol)	-16.3	-14.9	-14.7	-14.5	-14.3	-14.2	-14.2
Model	Model 8	Model 9	Model 10	Model 11	Model 12	Model 13	Model 14
Binding affinity (kcal/mol)	-14.2	-14.2	-14.1	-14.1	-14.1	-13.9	-13.9
Model	Model 15	Model 16	Model 17	Model 18	Model 19	Model 20	
Binding affinity (kcal/mol)	-13.9	-13.8	-13.7	-13.5	-13.5	-13.5	

4 Conclusion

In this study, we present free energy profile of ligand-CA I complex. A simple cluster model derived from the structure by X-ray analysis is used to estimate the force field of the zinc ion in the CA I active site. The force field parameters related to the zinc ion with MD simulation has been summarized. The free energy profile in relation to the binding/dissociation process of ligand from the CA I enzyme has been estimated by integration method combined with all-atom molecular dynamics simulation. From our simulations, we find that the binding free energy of ligand/CA I complex from the reference state becomes -53.11$\pm$ 14.17 kcal/mol and the free energy reaches the minimum at r_{cm} 8.5 Å. This distance r_{cm} is a good agreement with that of the equilibrium MD. On the other hand, we have performed all-atom MD simulations of plastocianin in complex with cytochrome f. We have shown that the distance between the plastocianin and cytochrome f obtained by our calculation has a good agreement with the equilibrium MD. To examine the detail of the association/dissociation process of Pc/Cyt$f$ complex, we have performed PaCS-MD simulation with similar procedure calculation presented in the thesis. From our results, we obtain that the time simulation of 3500-ps MD corresponding to 35 cycles of 10 MIMD for 100-ps is sufficient to generate the dissociation pathway of plastocianin from the side of cytochrome f. Meanwhile, in association pathway, we observe that plastocianin move into the region of cytochrome f because the distance between metals in the complex structure is similar with the X-ray structure. Also,

we have performed molecular docking of the 60S ribosomal subunit and the RA-VII molecule. Twenty models are obtained by molecular docking simulations. From those models, model 1 has the lowest binding affinity and may become a promising drug for anti-tumor.

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令和　2年　8月　3日

学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

Theoretical studies on association/dissociation process of protein complex related to protein-protein and ligand-receptor interactions （タンパク質―タンパク質相互作用及びリガンド―受容体相互作用に関連するタンパク質複合体の会合解離過程に関する理論的研究）

2．論文提出者　(1) 所　　属　数物科学　　専攻

(2) 氏（ふり）　名（がな）　Arwansyah Muhammad Saleh（あるわんさ　むはまど　されh）

3．審査結果の要旨（600～650字）

当該学位論文に関して、各審査員が個別に検討した後、令和２年７月２１日に予備審査を行い論文内容を詳細に検討した。その後令和２年８月３日に行われた学位論文公聴会の後に審査委員会を開き、協議の結果以下のように判定した。

Arwansyah Muhammad Saleh 氏は、タンパク質活性部位に亜鉛イオンを含む金属タンパク質活性部位付近の電子構造を解析し、タンパク質―タンパク質相互作用と関連させながらタンパク質とリガンドの会合解離過程を理論的に解析した。タンパク質とリガンドが複合体形成する過程に於いて、両者が近距離で相互作用することにより結合箇所構造あるいは配座を変化させて複合体が形成される誘導適合モデルが提唱されている。Arwansyah Muhammad Saleh 氏は鍵―鍵穴説と比べると誘導適合モデルの方が優位であることを示した。この結果は他のタンパク質―リガンド複合体形成シミュレーション研究を実施する場合でもタンパク質の柔軟さが重要であることを示唆する。この一連の研究結果は、タンパク質―リガンド複合体形成過程の計算による解析の有効性を示すと共に、リガンド―受容体相互作用に関わる今後の理論や実験研究にも多くの寄与をもたらすものである。以上により、この論文は博士（理学）に値するものと判断した。

4．審査結果　(1) 判　　定（いずれかに○印）（合　格）・　不合格

(2) 授与学位　　博　士（　理学　）