Theoretical studies on association/dissociation process of plastocyanin and cytochrome $f$ in photosynthesis

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Abstract. We investigate the association/dissociation process of plastocyanin with cytochrome $f$ before/after the reaction of the electron transfer from cytochrome $f$ to plastocyanin by using all-atom molecular dynamics simulation and our coarse-grained model simulation. The force field parameters of the oxidized and reduced plastocyanin are estimated by quantum chemical calculations and are summarized in this paper. The free energy profiles corresponding to the association/dissociation process are calculated by the thermodynamic integration method with the all-atom molecular dynamics simulations. A flat free energy landscape in the middle range of the association/dissociation process for both cases before and after the reaction is observed. The radial distribution function of the case before the reaction is calculated by the coarse-grained model with the Langevin equation of motion. We estimate the free energy landscape from the radial distribution function. The results by the coarse-grained model may reproduce similar results by all-atom molecular dynamics simulation. We discuss the association/dissociation process of the complex of plastocyanin and cytochrome $f$ in relation to the free energy landscape with some physical properties such as the binding free energy, the activation free energy, the energy of the reaction, and so on.

1. Introduction
Plastocyanin is one of the blue copper proteins which have the function of the electron transfer between proteins. The soluble protein of plastocyanin catches one electron from cytochrome $f$ in cytochrome $b_{6}f$ complex. The reduced plastocyanin moves in thylakoid lumen by diffusion and releases the electron to P700 in Photosystem I. The active site of plastocyanin consists of one copper ion, two histidines, one cysteine, and one methionine. The copper ion is coordinately bonded to two nitrogen atoms in the histidines, one sulfur atom in the cysteine, and the sulfur atom in the methionine. The structure of plastocyanin has been analyzed by X-ray analysis[1, 2, 3, 4]. On the other hand, the structure of cytochrome $f$ by X-ray analysis has been reported by C. J. Carrell and coworkers [5]. Cytochrome $f$ is one of the c-type cytochrome with a heme compound and has two soluble structural domains in the lumen-side segment. The structure of P700 has also been analyzed by X-ray analysis[6, 7].

The reduction and oxidization processes by the electron transfer are rapid with the complex between the soluble plastocyanin and the soluble domain in Cytochrome $f$ or the P700, and
the complex is short-lived and weak. The structure of the weak complex of plastocyanin has been investigated by many groups\[8, 9, 10, 11\]. The association and/or dissociation processes of plastocyanin has been discussed from the viewpoint of the hydrophobic and the electrostatic interactions between proteins in relation to the possible structures of the weak and short-lived complex\[10, 11\]. In our previous studies\[12, 13\], we have presented a simple coarse-grained (CG) model describing protein-protein interaction to investigate the structure and the dynamical properties such as the reaction rate of the electron transfer with protein complexes\[13\] and so on\[12\]. The reaction rate of the electron transfer of plastocyanin with cytochrome \(f\) has been investigated by our coarse-grained model\[13\]. We have found the results in the previous work is a good agreement with experimental one\[14\].

In this study, we focus the association/dissociation processes of plastocyanin with cytochrome \(f\) before and after the electron transfer reaction written as \(\text{PC}_{\text{ox}}-\text{Cyt}_{\text{red}} \rightarrow \text{PC}_{\text{red}}-\text{Cyt}_{\text{ox}}\), where \(\text{PC}_{\text{ox}}\) and \(\text{PC}_{\text{red}}\) mean plastocyanin of the oxidized and reduced states, respectively, and \(\text{Cyt}_{\text{red}}\) and \(\text{Cyt}_{\text{ox}}\) correspond to reduced and oxidized cytochrome \(f\), respectively. The association process of oxidized plastocyanin and reduced cytochrome \(f\) is investigated by all-atom molecular dynamics simulation to estimate the free energy profile corresponding to the association process. We also investigate the dissociation process of reduced plastocyanin and oxidized cytochrome \(f\) after the electron transfer by the same procedure of simulations. The force field parameters around the active site in reduced and oxidized states of two proteins for all-atom molecular dynamics simulations are estimated by quantum chemical calculations. We calculate the free energy profile by all-atom molecular dynamics simulation according to the procedure of the estimation of the free energy profile presented in the previous works\[15, 16\]. We discuss several snapshot structures of the complex between plastocyanin and cytochrome \(f\) in the association process by comparing with the results estimated by our coarse-grained model in relation to the stability of the complex with some physical properties such as the binding free energy, the activation energy of the reaction, the free energy of the reaction and so on.

2. Molecular dynamics simulations and coarse-grained modeling

The free energy profiles corresponding to two states before and after the electron transfer are calculated by all-atom molecular dynamics simulation with the thermodynamic integration method\[15, 16\] and by the coarse-grained model presented in the previous work\[13\].

2.1. Molecular dynamics simulation

The X-ray crystal structures of plastocyanin solved by Guss, et al. (PDB ID: 1PLC) \[17\] and the lumen-side domain of cytochrome \(f\) solved by Martinez, et al. (PDB ID: 1HCZ) \[18\] are used as the initial structures for the simulation of association process. These structures correspond to the oxidized plastocyanin and the reduced cytochrome \(f\), respectively. As for the dissociation process, the simulation was performed by using the NMR crystal structure of the complex of poplar plastocyanin with turnip cytochrome \(f\) solved by Lange, et al. (PDB ID: 1TKW) \[19\]. We adapted different crystal structure for association and dissociation processes because the oxidation state of plastocyanin and cytochrome \(f\) existed in those processes is different. In the association process, plastocyanin and cytochrome \(f\) were found in oxidized and reduced state, respectively, while in the dissociation process, as the reaction has occurred, plastocyanin and cytochrome \(f\) were found in reduced and oxidized state, respectively. Thus, we assumed that the initial structure of both proteins used in the simulation is similar to the natural system. Plastocyanin is a water-soluble protein, consists of 99 amino acid residues, and includes Cu\(^{2+}\) ion in its active site. The lumen-side domain of cytochrome \(f\) consists of 252 amino acid residues and includes a heme and Fe\(^{2+}\) in its active site. Plastocyanin and the lumen-side domain of cytochrome \(f\) are immersed in a water solvent.
The partial charge distribution around the active sites before and after the electron transfer process was calculated by using quantum calculations. The force field parameters related to the active sites of plastocyanin before and after the electron transfer process were also calculated by using quantum calculations. The quantum calculation was performed by using B3LYP density functional with 6-31G(d,p) basis set in Gaussian package [20]. More detailed procedures to calculate the force field parameters related to the active sites have been presented in Ref. [21].

As for the cytochrome \( f \), the force field parameters of the structure before and after the reaction are set to be similar to those of reduced cytochrome \( f \) presented by CHARMM27[22, 23]. However, note that in this simulation, we do not focus on the structural relaxation after the reaction of the electron transfer because we assume that the electron transfer occurred faster than the structural dynamics.

Temperature and pressure are controlled at 300 K by using Nose-Hoover chain and 0.101 MPa by using Andersen algorithm, respectively [24, 25, 26]. The long-range coulombic interactions under the periodic boundary condition are calculated by Particle Mesh Ewald method [27], in which the Ewald dispersion parameter is \( 0.375 \times 10^{10} \text{m}^{-1} \), 64 × 64 × 64 grids are adopted, and the cutoff distance is 1.2 nm. The Newton’s equations of motion are solved with the time step of 2.0 fs. The SHAKE/RATTLE/ROLL algorithm is used for constraint of the bond lengths including the hydrogen atoms [26]. The force field parameters are derived from CHARMM27 [22, 23] and TIP3P [28] for cytochrome \( f \) protein and water molecules, respectively. Molecular dynamics (MD) simulation was performed with a constraint on the distance between the mass center of plastocyanin and cytochrome \( f \) by using SHAKE and RATTLE methods. The constraint distance was gradually decreased and increased from \( r = 31.25 \text{Å} \) to 25 Å and 45 Å, respectively. The initial structures with 17 different distance were prepared, which the distance was varied from 25 Å to 45 Å with the interval of 1.25 Å.

Thermodynamic integration method [29] is applied to calculate the free energy profile as a function of the distance between the centers of mass of plastocyanin and cytochrome \( f \). We apply same procedure as our previous study [15] to calculate the free energy profile from the trajectories of molecular dynamics simulations. All molecular dynamics simulations are performed by using the MODYLAS program package [30].

### 2.2. Coarse-grained model

In our coarse-grained model, each amino acid residue is represented as one coarse-grained particle located on \( \alpha \) carbon. We solve the Langevin equation of motion of the \( i \)-th coarse-grained particle described by

$$
\frac{m_i}{d^2}\mathbf{r}_i = -\frac{\partial V}{\partial \mathbf{r}_i} - \gamma \frac{d\mathbf{r}_i}{dt} + \mathbf{P}_i(t),
$$

where \( m_i, \mathbf{r}_i, V \) are represented the mass and coordinate of the \( i \)-th coarse-grained particle, and the total potential energy of target system, respectively. We set the mass of coarse-grained particle 137 amu. The friction coefficient represented as \( \gamma \) does not depend on the amino acid residue and is set to \( 3.1 \times 10^{-13} \text{kg/s} \). The random force, \( \mathbf{P}_i(t) \), is the Gaussian white noise described by

$$
\langle P_{i\alpha}(t) \rangle = 0, \quad (\alpha = x, y, z),
$$

$$
\langle P_{i\alpha}(t)P_{j\beta}(t') \rangle = 2\gamma k_B T \delta_{ij} \delta_{\alpha\beta} \delta_{tt'},
$$

where \( P_{i\alpha}(t) \) indicates the \( \alpha \) component of random force at time \( t \). The total potential energy \( V \) is expressed as

$$
V = V_{\text{intra}} + V_{\text{inter}},
$$

where \( V_{\text{intra}} \) and \( V_{\text{inter}} \) indicate the intramolecular potential energy and the intermolecular potential energy, respectively. The intramolecular potential energy is used Gō-like potential [31]
described by

\[ V_{\text{intra}} = \sum_{\text{bonds}} K_b(r_i - r_{i0})^2 + \sum_{\text{angles}} K_\phi(\theta_i - \theta_{i0})^2 + \sum_{\text{dihedrals}} K_\psi \{1 - \cos(\phi_i - \phi_{i0})\} + \frac{1}{2} \{1 - \cos 3(\phi_i - \phi_{i0})\} \]

\[ + \sum_{i>j+3} \varepsilon_{\text{nc}} \left[ 5 \left( \frac{r_{ij0}}{r_{ij}} \right)^2 - 6 \left( \frac{r_{ij0}}{r_{ij}} \right)^10 \right] + \sum_{i>j+3} \varepsilon_{\text{nnc}} \left( \frac{d - r_{ij}}{r_{ij}} \right)^2. \]  

(5)

In the equation (5), \( r_i, \theta_i, \phi_i, r_{ij} \) represent the \( i \)-th virtual bond length between \( i \)-th and \((i+1)\)-th coarse-grained particles, the virtual bond angle between \( i \)-th and \((i+1)\)-th virtual bonds, the virtual dihedral angle around the \((i+1)\)-th virtual bond, and the distance between \( i \)-th and \( j \)-th coarse-grained particles, respectively. The subscript "0" means the value at the reference structure. In the fourth and fifth term, \( \text{nc} \) and \( \text{nnc} \) indicate native contact and non-native contact, respectively. The other parameters are constant values and are set to \( K_b = 100 \text{kcal/mol} \cdot \AA^2 \), \( K_\phi = 20 \text{kcal/mol} \cdot \text{rad}^2 \), \( K_\psi = 1 \text{kcal/mol} \), \( \varepsilon_{\text{nc}} = \varepsilon_{\text{nnc}} = 3.6 \text{kcal/mol} \), and \( d = 4.0 \AA \).

The intermolecular potential energy proposed in our previous study[13] consists of four terms described by

\[ V_{\text{inter}} = V_{\text{LJ}} + V_{\text{DP}} + V_G + V_{\text{rep}}. \]  

(6)

In the equation (6), \( V_{\text{LJ}}, V_{\text{DP}}, V_G, \) and \( V_{\text{rep}} \) mean the van der Waals interaction between two hydrophobic amino acid residues in different protein molecules, the depletion interaction, two Gaussian potentials, and the repulsive potential between two coarse-grained particles which do not correspond to hydrophobic amino acid residues, respectively. These potential energies are written as

\[ V_{\text{LJ}} = \sum_{i \alpha} \varepsilon_{LJ}^{i \alpha} \left[ \left( \frac{R_{ia}}{r_{ia}} \right)^{12} - 2 \mu(\rho_i) \mu(\rho_\alpha) \left( \frac{R_{ia}}{r_{ia}} \right)^6 \right], \]  

(7)

\[ V_{\text{DP}} = -\frac{\pi \rho_0}{12} \sum_{i \alpha} \mu(\rho_i) \mu(\rho_\alpha) (r_{ia} - l_{ia})^2 (r_{ia} + 2l_{ia}), \]  

(8)

\[ V_G = \sum_{k=1}^2 \sum_{i \alpha} \mu(\rho_i) \mu(\rho_\alpha) \varepsilon_{G(k)}^{i \alpha} \exp \left[ -\left( \frac{r_{ia} - \lambda_{ia}^{(k)}}{\sigma_{ia}^{(k)}} \right)^2 \right], \]  

(9)

\[ V_{\text{rep}} = \sum_{i,\alpha \neq \text{hydrophobic}} \varepsilon_{\text{rep}} \left( \frac{C}{r_{ia}} \right)^{12}, \]  

(10)

where \( r_{ia} \) represents the distance between the \( i \)-th and \( \alpha \)-th coarse-grained particles in different protein molecules. The osmotic pressure, \( \rho_0 \) is estimated by all-molecular dynamics simulation and set to 1.37974 \times 10^8 \text{ Pa} in this work. \( \varepsilon_{LJ}^{i \alpha}, R_{ia}, \varepsilon_{G(k)}^{i \alpha}, \lambda_{ia}^{(k)}, \) and \( \sigma_{ia}^{(k)} \) are parameters obtained by all-molecular dynamase simulations and satisfy with the Lorentz-Berthelot mixing rules[32]. In the equation (9), \( l_{ia} \) is described by \( l_{ia} = (R_{ia} + R_W)/2 \). \( R_W \) represents van der Waals parameter and is set to 1.4\AA. In the equation (10), \( \varepsilon_{\text{rep}} \) and \( C \) are constant parameters and are given as 3.6 kcal/mol and 4.0\AA, respectively.

We introduce a function related to the molecular crowding effect as

\[ \mu(\rho_i) = \frac{1}{\exp \left[ 20(\frac{\rho_i}{\rho_i^c} - 1) \right] + 1}. \]  

(11)
where $\rho^*$, $\rho_i$ is the critical density of hydrophobic amino acid residue and is the $i$-th density of hydrophobic amino acid residue in different protein molecules, respectively. $\rho_i$ is expressed as

$$\rho_i = \sum_{\alpha} \frac{1}{\exp\left[\frac{20(\frac{r_{i\alpha}}{\sigma} - 1)}{\sigma}\right] + 1},$$  \hspace{1cm} (12)$$

where $\sigma$ corresponds to the constant parameter with a distance dimension. We set $\rho^*$ and $\sigma$ to 6.2 and 13.9Å from our previous study[13]. If the density of particle is higher than the critical density, the attractive term of $V_{\text{LJ}}$, $V_{\text{DP}}$, and $V_G$ rapidly become zero.

3. Results and discussion

Table 1 summarizes force field parameters for bonds around the copper ion in the active site of oxidized and reduced states of plastocyanin derived by quantum mechanical calculations. $r_c$ and $K_r$ mean the equilibrium distance and the force constant for the bond stretching, respectively. The bond stretching potential $U_b(r)$ can be expressed as $U_b(r) = K_r(r - r_c)^2$, where $r$ is the distance between two atoms. Other parameters for the angle of plastocyanin are summarized in Table 2. $\theta_c$ and $K_\theta$ correspond to the equilibrium angle and the force constant for the angle bending, respectively. The potential energy for the angle bending can be expressed as $U_a(\theta) = K_\theta(\theta - \theta_c)^2$, where $\theta$ is the angle bending defined by three atoms. We use the parameters summarized in those Tables for all-atom molecular dynamics simulations. Figure 1 and 2 show schematic diagrams of the charge distributions of the oxidized and the reduced plastocyanin, respectively. The schematic diagrams of the charge distribution of the active site of the oxidized and reduced cytochrome $f$ proteins are also shown in Figs. 3 and 4, respectively.

![Figure 1. Charge distribution around the active site of oxidized plastocyanin.](image)

![Figure 2. Charge distribution around the active site of the reduced plastocyanin.](image)

Figure 5 shows the free energy profile of the oxidized plastocyanin with the reduced cytochrome $f$. The horizontal axis in Fig. 5 means the distance between the centers of mass of the protein. From Fig. 5, we can approximately estimate the binding free energy of the state before the electron transfer reaction as about 19.5 kcal/mol, where we assume the free energy value at a distance between the centers of mass of 45Å is the origin of the free energy. The flat free energy landscape is observed around the region from 35Å to 40Å. In the flat free energy landscape, we can find the extremely small activation energy as 1.8 kcal/mol around 35Å in Fig. 5. Figure 6 shows the free energy profile of the state after the electron transfer reaction. The
**Figure 3.** Charge distribution around the active site of oxidized cytochrome f.

**Figure 4.** Charge distribution around the active site of the reduced cytochrome f.

**Figure 5.** Free energy profile corresponding to the association process before the reaction of the electron transfer.

**Figure 6.** Free energy profile corresponding to the dissociation process after the reaction of the electron transfer.

**Table 1.** Parameters for bonds around the active site.

|                | Oxidized State | Reduced State |
|----------------|----------------|---------------|
|                | $r_c$ [Å] | $K_r$ [kcal/mol/Å$^2$] | $r_c$ [Å] | $K_r$ [kcal/mol/Å$^2$] |
| Cu-N(His37)    | 1.975  | 110.817        | 1.94   | 117.21        |
| Cu-S(Cys84)    | 2.145  | 138.055        | 2.20   | 100.95        |
| Cu-N(His87)    | 2.021  | 87.92          | 2.09   | 71.91         |
| Cu-S(Met92)    | 2.988  | 26.146         | 2.82   | 17.41         |
Table 2. Parameters for angles around the active site.

|                        | Oxidized State | Reduced State |
|------------------------|----------------|---------------|
|                        | $\theta_c$ [degree] | $K_\theta$ [kcal/mol/rad$^2$] | $\theta_c$ [degree] | $K_\theta$ [kcal/mol/rad$^2$] |
| N(His37)-Cu-S(Cys84)   | 130.542        | 285.948       | 136.29          | 237.6          |
| N(His37)-Cu-N(His87)   | 96.491         | 349.708       | 99.91           | 214.46         |
| N(His37)-Cu-S(Met92)   | 87.075         | 307.703       | 87.26           | 312.8          |
| S(Cys84)-Cu-N(His87)   | 134.434        | 69.157        | 108.95          | 120.85         |
| S(Cys84)-Cu-S(Met92)   | 111.733        | 294.284       | 114.22          | 249.35         |
| N(His87)-Cu-S(Met92)   | 107.425        | 55.564        | 106.02          | 199.13         |
| Cu-N(His37)-C$_\epsilon$ | 122.682   | 245.728       | 122.99          | 81.53          |
| Cu-N(His37)-C$_\gamma$ | 127.68        | 224.148       | 120.71          | 92.79          |
| Cu-S(Cys84)-C$_\beta$  | 113.062        | 74.816        | 106.18          | 36.27          |
| Cu-N(His87)-C$_\epsilon$ | 120.787   | 88.285        | 128.57          | 208.46         |
| Cu-N(His87)-C$_\gamma$ | 95.602         | 88.196        | 117.95          | 225.52         |
| Cu-S(Met92)-C$_\epsilon$ | 97.66     | 62.314        | 98.37           | 106.77         |
| Cu-S(Met92)-C$_\gamma$ | 127.663        | 253.084       | 133.52          | 195.27         |

Figure 7. The snapshot of a structure around the region of the lowest free energy. The secondary structure plastocyanin and cytochrome $f$ represent by the structure with the color of violet and green, respectively.

Figure 8. The snapshot of a structure around the distance 35Å of the flat free energy landscape in the association process. The secondary structure plastocyanin and cytochrome $f$ represent by the structure with the color of violet and green, respectively.

The binding free energy is estimated as 23.9 kcal/mol, and we can find the flat free energy landscape around the region from 35Å to 40Å similar to that shown in Fig. 5. However, we can not
Figure 9. The snapshot of a structure around the distance 40Å of the flat free energy landscape. The secondary structure plastocyanin and cytochrome $f$ represent by the structure with the color of violet and green, respectively.

Figure 10. Close view of the binding region of the complex between the oxidized plastocyanin and the reduced cytochrome $f$ of the snapshot shown in Fig. 7.

Figure 11. Close view of binding region of the complex between the reduced plastocyanin and the oxidized cytochrome $f$ around the lowest free energy at the distance about 25Å shown in Fig. 6.

find the certain activation energy in the case of the dissociation process between the reduced plastocyanin and the oxidized cytochrome $f$.

Free energy profile shown in Figs. 5 and 6 is calculated as a function of the distance between the centers of mass of two protein molecules by using the thermodynamic integration (TI) method. Even though their orientation also has an impact on the free energy profile, it is calculated as a function of only the distance by averaging the motion of the orientation. The detail procedures have been reported in our previous studies [15]. Calculated binding free
energy is around 20 kcal/mol for both cases. Although it seems to be larger than expected binding free energy, the binding free energy along the distance could be around 20 kcal/mol or more[15, 33, 37]. The free energy profile does not become constant at long range of the distance between those proteins because plastocyanin may not leave the surface of cytochrome $f$ at 45Å.

From the two results shown in Figs. 5 and 6, the free energy of the reduction reaction of plastocyanin becomes about 4.4 kcal/mol. Because no crossing between two free energy landscapes, we can approximately expect the electron transfer reaction from the reduced cytochrome $f$ to the oxidized plastocyanin proceeds soon once the complex is formed. The implicit result of the rapid reaction presented in this work is good agreement with the comments by other works[14, 34, 35, 36].

Figures 7, 8, and 9 show some snapshots corresponding to the configurations in the association process of the oxidized plastocyanin and the reduced cytochrome $f$. In those figures, the structure of plastocyanin and cytochrome $f$ was represented by the structure with violet and green colors, respectively. A snapshot structure of the complex around the lowest free energy at a distance about 26Å is shown in Fig. 7. The configuration is similar to the crystal structure analyzed by X-ray analysis. The snapshot structures shown in Fig. 8 and 9 are sometimes found in the flat free energy landscape with the distances about 35Å and 40Å, respectively. The configuration shown in Fig. 8 roughly changes to that in Fig. 9 with keeping the distance between the two surfaces.

Figures 10 and 11 show close views for the binding site of the complex structures before and after the reaction around the lowest free energy, respectively. Similar to the previous figures, the structure with violet and green colors represent the structure of plastocyanin and cytochrome $f$, respectively. From these figures, we can find the distance between two active sites is close in the case of the lowest free energy and can find two regions consisting of hydrophobic and acid patches. In the hydrophobic patch, there are Ala90, Pro86, and Pro36 in plastocyanin binding with Phe4, Ile3 in cytochrome $f$ shown in Fig. 10. On the other hand, in the case after the electron transfer, the distance between the active sites becomes shorter than that before the reaction. These results imply the hydrophobic interaction between the active sites contributes to the stability of the configuration of the complex of plastocyanin and cytochrome $f$.

Let us discuss the results obtained from the coarse-grained model presented in the previous work[13]. Figure 12 shows the radial distribution function of the distance between the centers of mass. The peak of the radial distribution function is around 27Å. The distance between the centers in the complex structure by NMR analysis is about 26.1Å (PDB ID: 2PCF)[8]. The result suggests that cytochrome $f$ and plastocyanin have the structure similar to that by experiments and that the structure obtained by the present simulation is a good agreement with the experimental result[8].

The solid line shown in Fig. 13 shows the free energy landscape obtained from the radial distribution function shown in Fig. 12. The vertical axis in Fig. 13 is plotted with the renormalization by the binding free energy. The dashed line means the free energy profile from Fig. 5. The lowest free energy value is observed around the distance 27Å, and we can find the flat free energy landscape around the region from 35Å to 40Å. The flat free energy landscape obtained from the coarse-grained model may become similar to that obtained by all-atom molecular dynamics simulations shown in Fig. 5. On the other hand, from Fig. 13, it is not easy to find the activation energy of the reaction similar to that shown in Fig. 5.

We can find the large difference of the free energy value around shorter distance than the region around the lowest free energy by the coarse-grained model on that by the molecular dynamics simulation from Fig. 13. To compare the landscape by the coarse-grained model with that by all-atom molecular dynamics simulation with better accuracy, it may be necessary to take a larger number of samplings. It may also be better to multiply the binding free energy, obtained by a coarse-grained model, by a constant as the ratio of the total number of the freedom of the
coarse-grained particle to that of the solute of protein. This is necessary to directly compare the result by the coarse-grained model with that of all-atom molecular dynamics simulation. The at least shape of the free energy landscape obtained from the coarse-grained model corresponding to the dissociation process may become similar to that obtained by all-atom molecular dynamics simulations.

Figure 12. Radial distribution function of the distance between the centers of the oxidized plastocyanin and the reduced cytochrome f.

Figure 13. Free energy landscape obtained from Fig. 12, which the error bar represent the standard deviation of the free energy obtained from all-atom simulation.

4. Summary
In this study, we have investigated the association/dissociation processes of plastocyanin with cytochrome f before/after the reaction of the electron transfer from cytochrome f to plastocyanin by using all-atom molecular dynamics simulations and our coarse-grained model simulations.

The force field parameters around the active sites of the oxidized and reduced plastocyanin have been estimated by quantum chemical calculations and have been summarized in this paper. The free energy profiles corresponding to the association/dissociation processes have been calculated by the thermodynamic integration method with the all-atom molecular dynamics simulations. From the simulations, we have found a flat free energy landscape in the middle range of the association/dissociation processes for both cases before and after the reaction. The results suggest that in this flat free energy landscape, plastocyanin dissociates from cytochrome f with gradually changing the conformation of the complex while rotating and keeping the distance between the surfaces of the proteins. The conformation changing in the dissociation process has also been theoretically pointed out by Gumbart etc. [37].

We have also found the small activation free energy about 1.8 kcal/mol in the case before the reaction at a distance between the centers of mass of the proteins around 35Å. However, there is no activation free energy after the reaction. The results from the crossing the free energy profiles for both cases before and after the reaction suggest the reaction of the electron transfer proceeds soon once the complex of the oxidized plastocyanin and the reduced cytochrome f is formed. The binding free energy, the activation energy, and the free energy of the reaction have been estimated by all-atom molecular dynamics simulations. The binding free energies before and after the reactions are about 19.5 kcal/mol around 26Å and about 23.9 kcal/mol around 25Å. Therefore, the free energy of the reaction becomes 4.4 kcal/mol.

The radial distribution function of the case before the reaction has been calculated by the coarse-grained model with the Langevin equation of motion. We have estimated the free energy
landscape corresponding to the association/dissociation processes from the radial distribution function and have found the flat free energy landscape in the region from 35 Å to 45 Å similar to that obtained by the all-atom molecular dynamics simulation. The results by the coarse-grained model may be a good agreement with that by all-atom molecular dynamics simulation. We will present more accurate data by a larger number of sampling for simulations elsewhere [38].

Finally, we have calculated the free energy profiles for both cases before and after the reaction of the electron transfer from reduced cytochrome \( f \) to oxidized plastocyanin by all-atom molecular dynamics simulations and the coarse-grained model simulations. We have also discussed the association and/or dissociation processes of the complex of plastocyanin and cytochrome \( f \) in relation to the free energy landscape with some physical properties such as the binding free energy, the activation free energy, the energy of the reaction, and so on. The flat free energy landscape has been found in the middle range of the association/dissociation processes.

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