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Analysis of Interactions between Luciferase and Si Substrates Using Molecular Dynamics Simulations

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A series of molecular dynamics (MD) simulations have been performed to investigate the interactions between luciferase and Si substrates. The results show that luciferase adsorbs directly on the hydrophobic Si substrate, and via water molecules on the hydrophilic one. The adsorption-induced changes in conformation of luciferase are smaller on the hydrophilic Si substrate than on the hydrophobic one. The dynamic atom motions in luciferase are larger on the hydrophilic Si substrate than on the hydrophobic one. Inside the active site, the adsorption-induced changes in distances between the atoms forming hydrogen bonds to substrate luciferin are smaller on the hydrophilic Si substrate than the hydrophobic one. In order to prevent the denaturation of luciferase caused by immobilization, the solid surface should be hydrophilic. For higher thermostability, after immobilization, however, a hydrophobic surface is preferable since the dynamic atom motions in luciferase are smaller on a hydrophobic surface. The solid surface should be prepared delicately both from the viewpoint of preventing the denaturation caused by immobilization and improving the thermostability. [DOI: 10.1143/JJAP.45.1021]

KEYWORDS: luciferase, Si substrates, nanostructure, molecular dynamics simulations, MD, biomedical application, protein chip

1. Introduction

Interactions between proteins and solid surfaces are of interest for biomedical applications such as the adenosine triphosphate (ATP) sensor, the ATP synthesizer and immunoassays.[1–3)] For example, the ATP sensor is fabricated by immobilizing firefly luciferase on a Si surface, the ATP synthesizer by immobilizing ATPase on a glass surface, and immunoassays by immobilizing immunoglobulin G (IgG) on a Si surface. These applications are based on the technique of immobilizing proteins on solid surfaces. The immobilization technique is also important for *in vitro* analysis of cells, for example, the estimation of drug efficacy with respect to a specific protein in a cell culture.

The denaturation of immobilized proteins is a serious problem when proteins are immobilized on solid surfaces. In some studies, proteins were linked on solid surfaces via self-assembled monolayers (SAMs), His-tag, or other means in order to avoid denaturation.[4–7)] Some experimental trials have suggested the possibility of immobilizing proteins without denaturation even for a direct contact between proteins and solid surfaces.[1,2)] The compatibility of a solid surface with the target proteins is critically important to avoid denaturation when the proteins adsorb on solid surfaces.

Si surfaces with a nanofabricated and wet-treated array may achieve compatibility with proteins for the following two reasons. Current Si-process technology enables us to fabricate a nanostructure array on Si substrates.[8,9)] A delicate control of the surface conditions, such as hydrophobic or hydrophilic properties, is also possible due to the recent progress in wet treatment technology for Si. The fine design of the fabrication process requires systematic information about interactions between proteins and solid surfaces; however, these interactions are not clearly understood.

Changes in conformation of proteins or changes in dynamic atom motions in proteins are the key indicators of denaturation. Denaturation is a state in which proteins lose this original activity due to an inappropriateness in their shape or in their environment. Protein denaturation becomes prominent when changes in conformation and dynamic atom motions become too large.

The purpose of our study is to clarify the immobilization mechanism of a protein on an artificial surface and to understand the reason for the denaturation caused by immobilization. In our previous work, we analyzed interactions between green fluorescent protein (GFP) and Si substrates using molecular dynamics (MD) simulations.[10)] In this work, we perform MD simulations for a system composed of luciferase and a Si substrate, because luciferase has broad prospective applications and considerable knowledge has been accumulated on its experimental handling. As the Si substrates, we have prepared a hydrogen-terminated Si substrate as an example of a hydrophobic Si substrate and a hydroxy-terminated Si substrate as a hydrophilic Si substrate.

Here we report adsorption states of luciferase on Si substrates, adsorption-induced changes in luciferase, and surface conditions suitable for immobilization of luciferase.

2. Calculation Method

The MD simulations on the luciferase-Si substrate systems are carried out using the program AMBER 7.0.[11)] Structural data for luciferase are derived from the Protein Data Bank (PDB) (PDB code: 1ba3). The hydrogen-terminated Si substrate consists of 992 Si atoms and 256 H atoms. The substrate is 5 Å thick in the [001] direction and 57 Å wide and long in $[1\bar{1}0]$ and [110]. The hydroxy-terminated Si substrate consists of 1023 Si atoms, 640 O atoms and 257 H atoms. The substrate is 7 Å thick in [001] and 57 Å long in $[1\bar{1}0]$ and [110]. During the simulations, atomic configurations of these Si substrates are fixed. In the initial structures, a luciferase surface area with relatively high hydrophobicity is placed facing the Si substrate.

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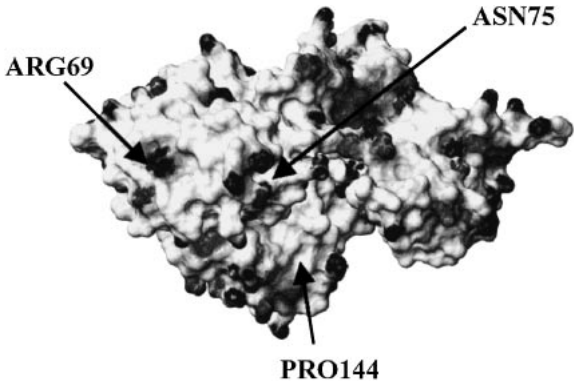

Fig. 1. Luciferase surface area with relatively high hydrophobicity (white: hydrophobic area, black: hydrophilic area).

Table I. Distances between residues and Si substrates in initial structure in units of Å.

	Hydrogen-terminated	Hydroxy-terminated
ARG69	5.8	6.0
ASN75	3.7	3.5
PRO144	5.6	5.8

Figure 1 shows the luciferase surface area with relatively high hydrophobicity. Table I shows the distances between the residues and the Si substrate in the initial structures. The luciferase-hydrogen-terminated Si substrate system is solvated with 15433 TIP3P water molecules[12] in a cubic box. The luciferase-hydroxy-terminated Si substrate system is solvated with 17170 water molecules in a cubic box.

The partial atomic charges of the Si substrates are determined on the basis of Hartree–Fock molecular orbital calculations on a small cluster model using the program Gaussian 98.[13] These partial atomic charges are calculated by Mulliken population analysis using small cluster models. For the hydrogen-terminated Si substrate, we use a cluster model consisting of 10 Si atoms and 16 H atoms, which is made by substituting C atoms for Si atoms in the adamantane structure (see Appendix A of ref. 10). For the hydroxy-terminated Si substrate, we use a small cluster model consisting of 10 Si atoms, 28 O atoms and 16 H atoms. The small cluster model is made by inserting O atoms into each bond of the small cluster model of the hydrogen-terminated Si substrate. The 6-31G** basis set is employed for these molecular orbital calculations. The force field of the Si substrates is determined using fast parameterization based on first-principles calculations.[14] These force field parameters are shown elsewhere.[10]

A three-dimensional periodic boundary condition is adopted, and the pressure and temperature are kept constant using the Berendsen algorithm.[15] The long-range electrostatic interactions are calculated by the particle mesh Ewald (PME) method.[16] The integration time step of the MD simulations is set to 1 fs.

The procedure for our simulations is as follows. First, the initial structures are optimized by potential energy minimizations (3500 steps of the steepest descent and 1500 steps of the conjugate gradient method). Next, MD simulations are performed from the minimized structures. Since the minimized structures are a kind of frozen structures at 0 K, the temperature of the systems is gradually increased from 5 to 300 K in 52 ps and then kept at 300 K.

Adsorption energies between luciferase and Si substrates are estimated with MM-PBSA modules[17] in AMBER 7.0. In MM-PBSA, adsorption energy is defined by the following equation:

$$\Delta G = G_{\text{complex}} - G_{\text{protein}} - G_{\text{substrate}}, \tag{1}$$

where G_{complex} is the free energy of the protein–Si substrate system, G_{protein} is the free energy of the isolated protein, and $G_{\text{substrate}}$ is the free energy of the isolated Si substrate.

We analyze the dynamic atom motions in the protein using the mass-weighted B-factor in AMBER 7.0. The mass-weighted B-factor is defined for each residue by the following equation:

$$\begin{aligned} &\sum_{a=N_{\text{res}}}^{N_{\text{res}+1}-1} m_a B_a \Big/ \sum_{a=N_{\text{res}}}^{N_{\text{res}+1}-1} m_a, \\ &B_a = \frac{8}{3}\pi^2 \langle \Delta r_a^2 \rangle, \\ &\Delta r_a = r_a - \langle r_a \rangle, \end{aligned} \tag{2}$$

where a is a label for atom number, res is for residue number, N_{res} is the number of the first atom in the residue res, m_a is the mass of each atom, and r_a is the coordinate of each atom.

3. Results

First we carry out the MD simulation of the luciferase–hydrophobic Si substrate system. The simulation is performed for 2000 ps. The potential energies decrease within 1500 ps from the beginning of the simulation, and then converge. Hence we define the time-averaged structure over the time after 1500 ps as a stable structure. Table II shows the distances between the residues and the hydrophobic Si substrate. From the reduction in these distances in the stable structure, we find that the luciferase spontaneously adsorbs on the hydrophobic Si substrate.

Next we carry out MD simulation of the luciferase–hydrophilic Si substrate system. The potential energies decrease within 1500 ps and then converge, as in the luciferase–hydrophobic Si substrate system. Table III shows the distances between the residues and hydrophilic Si

Table II. Distances between residues and hydrophobic Si substrate in units of Å.

	Initial	Stable
ARG69	5.8	4.1
ASN75	3.7	1.9
PRO144	5.6	2.1

Table III. Distances between residues and hydrophilic Si substrate in units of Å.

	Initial	Stable
ARG69	6.0	4.3
ASN75	3.5	1.9
PRO144	5.8	2.5

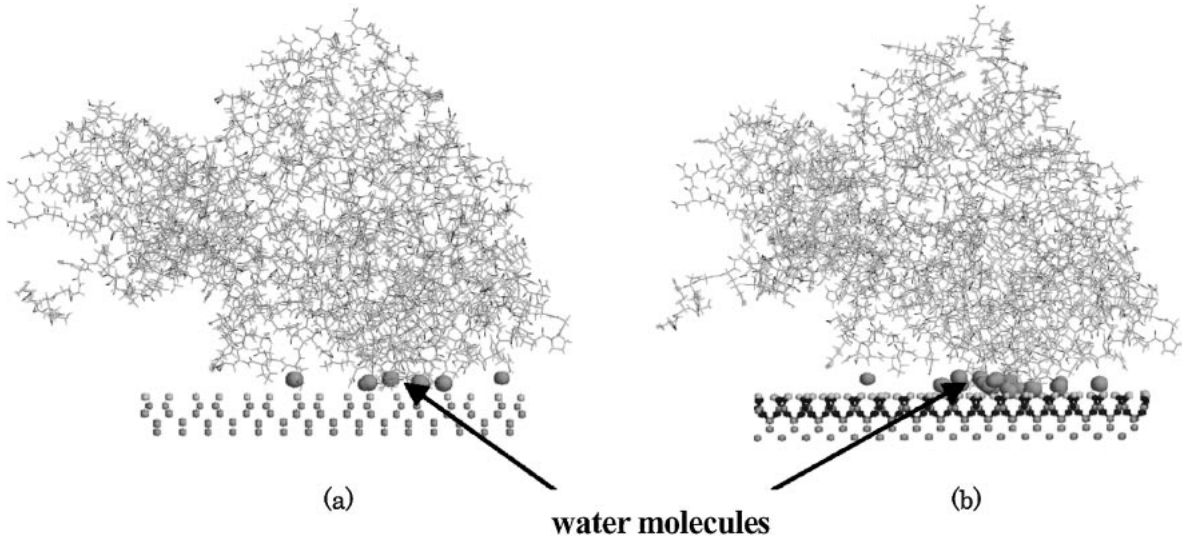

Fig. 2. Water molecules (highlighted) (a) between luciferase and hydrophobic Si substrate and (b) between luciferase and hydrophilic one in stable structure.

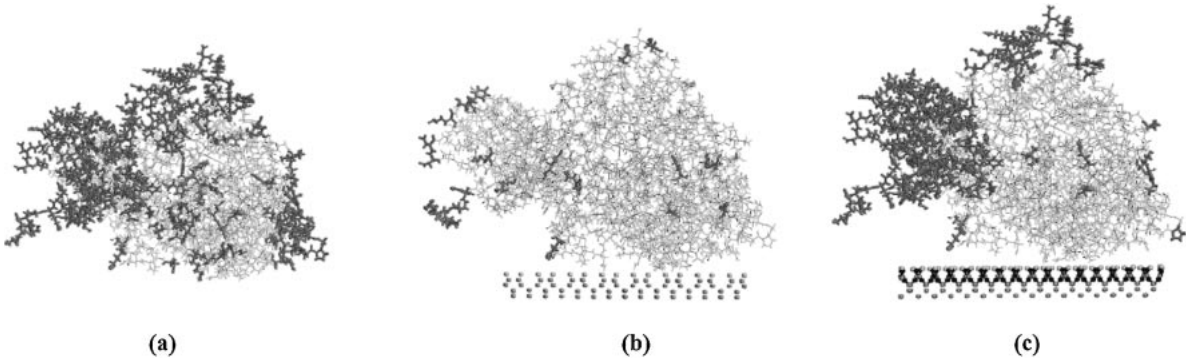

Fig. 3. Residues (highlighted) with B-factors over 40 Å^2 in luciferase (a) when isolated, (b) adsorbed on hydrophobic substrate, and (c) adsorbed on hydrophilic Si substrate.

substrate. From the reduction in these distances in the stable structure, we find that luciferase spontaneously adsorbs on the hydrophilic Si substrate, as on the hydrophobic Si substrate.

Figure 2 shows the stable structures with water molecules located between luciferase and the Si substrates. There are 6 water molecules between luciferase and the hydrophobic Si substrate; in contrast, 15 water molecules appear between luciferase and the hydrophilic one. This result shows that luciferase adsorbs directly on the hydrophobic Si substrate, and via water molecules on the hydrophilic one. The adsorption energies are estimated to be -61.1 kcal/mol for the hydrophobic Si substrate, and -32.3 kcal/mol for the hydrophilic one. This result shows that luciferase adsorbs more strongly on the hydrophobic Si substrate than on the hydrophilic one.

We analyze changes in conformation of luciferase induced by the adsorption on the Si substrate. The changes in conformation are estimated by the root mean square deviation (RMSD) value of their stable structures from the stable structure of luciferase$_{\text{isolated}}$ (luciferase isolated in water). The RMSD value of the whole luciferase structure is 2.38 Å in luciferase$_{\text{hydrophobic}}$ (luciferase adsorbed on a hydrophobic Si substrate) and 2.30 Å in luciferase$_{\text{hydrophilic}}$ (luciferase adsorbed on a hydrophilic Si substrate). Since the active site (residues 218, 246, 247, 251, 315, 341, 342, 343, 347, 348, 351, and 529) is important for activities of luciferase, the RMSD value of the active-site-surrounding residues is further examined. These estimated RMSDs are 2.08 Å in luciferase$_{\text{hydrophobic}}$ and 1.18 Å in luciferase$_{\text{hydrophilic}}$. The adsorption-induced changes in conformation of luciferase are smaller on the hydrophilic Si substrate than on the hydrophobic one in both the whole and the active site structure. This difference is prominent, especially at the active site. Accordingly, luciferase$_{\text{hydrophilic}}$ is preferable to luciferase$_{\text{hydrophobic}}$ in terms of keeping higher activity.

Next we analyze changes in the dynamic atom motions in luciferase induced by adsorption on the Si substrate. The magnitude of dynamic atom motions is estimated by the mass-weighted B-factors for each residue. Figure 3 shows residues with B-factors larger than 40 Å^2 in luciferase. The number of residues with B-factors larger than 40 Å^2 is 195 in luciferase$_{\text{isolated}}$, 23 in luciferase$_{\text{hydrophobic}}$, and 137 in luciferase$_{\text{hydrophilic}}$. This result shows a reduction in the dynamic atom motions in luciferase induced by the

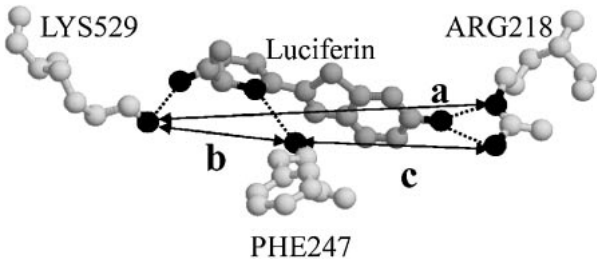

Fig. 4. Hydrogen bonds (dashed line) between luciferase and luciferin. Atoms forming hydrogen bonds are shown as black circles. Distances between atoms of ARG218, PHE247, and LYS529 forming hydrogen bonds to luciferin are defined as a, b and c, respectively.

Table IV. Distances between atoms of ARG218, PHE247, and LYS529 forming hydrogen bonds to luciferin when isolated and when adsorbed on hydrophobic and hydrophilic Si substrates in units of Å.

	Isolated	Hydrophobic	Hydrophilic
a	15.8	22.3	18.9
b	9.8	17.9	13.1
c	8.2	8.5	8.3

adsorption on the Si substrate. The mean B-factor of the whole structure is 38.85 Å^2 in luciferase$_{isolated}$, 26.20 Å^2 in luciferase$_{hydrophobic}$, and 32.53 Å^2 in luciferase$_{hydrophilic}$. The mean B-factor of the active site structure is 29.80 Å^2 in luciferase$_{isolated}$, 20.14 Å^2 in luciferase$_{hydrophobic}$, and 24.90 Å^2 in luciferase$_{hydrophilic}$. The dynamic atom motions in luciferase are larger on the hydrophilic Si substrate than on the hydrophobic one in both the whole and the active site structure. Accordingly, luciferase$_{hydrophilic}$ is preferable to luciferase$_{hydrophobic}$.

Luciferase catalyzes the ATP-dependent oxidation of luciferin, and the oxidized luciferin emits luminescence. Some atoms of ARG218, PHE247, and LYS529 form hydrogen bonds to luciferin.[18] The catalytic activity of luciferase depends on the distances between these atoms. We define the distances between these atoms as shown in Fig. 4. Table IV shows mean values of these distances. From Table IV, we find that the adsorption-induced changes in these distances are smaller on the hydrophilic Si substrate than on the hydrophobic one. Accordingly, luciferase$_{hydrophilic}$ is again preferable to luciferase$_{hydrophobic}$.

4. Discussion

For applications to biosensors or bioreactors, the immobilized proteins must retain a sufficiently high bioactivity. It is known that conformation of proteins and dynamic atom motions in proteins influence their functions. The adsorption-induced changes in conformation of luciferase are smaller on the hydrophilic Si substrate than on the hydrophobic one. The dynamic atom motions in luciferase are larger on the hydrophilic Si substrate than on the hydrophobic one. Inside the active site, the adsorption-induced changes in the distances between the atoms forming hydrogen bonds to luciferin are smaller on the hydrophilic Si substrate than on the hydrophobic one. From the viewpoint of the orientation of the luciferase on a Si substrate, the association of luciferin to luciferase is possible since the active site of luciferase is not covered by the Si substrate in this adsorption state. From these results, we conclude that the solid surface should be hydrophilic in order to prevent the denaturation of luciferase caused by immobilization.

The adsorption-induced changes in conformation of luciferase are smaller on the hydrophilic Si substrate than on the hydrophobic one. We explain this phenomenon on the basis of the difference in adsorption state, such as the number of water molecules as an intervening layer between luciferase and the Si substrates. The conformation of luciferase is affected by the contact area between luciferase and the Si substrates. Water molecules always appear between luciferase and the Si substrates since even the luciferase surface with relatively high hydrophobicity has not only hydrophobic areas, but also hydrophilic areas. The contact area between luciferase and the Si substrate is smaller on the hydrophilic Si substrate than on the hydrophobic one because there are 15 water molecules between luciferase and the hydrophilic Si substrate, and 6 water molecules between luciferase and the hydrophobic one. Accordingly, the adsorption-induced changes in conformation of luciferase are smaller on the hydrophilic Si substrate.

The dynamic atom motions in luciferase are larger on the hydrophilic Si substrate than on the hydrophobic one. We explain this phenomenon on the basis of the adsorption energy between luciferase and the Si substrate. The adsorption energies are estimated to be -61.1 kcal/mol for the hydrophobic Si substrate, and -32.3 kcal/mol for the hydrophilic one. The reason for the larger dynamic atom motions in luciferase on the hydrophilic surface is the weaker adsorption of luciferase.

On the other hand, in our previous work, we reported that the thermostability of a protein would be improved if the dynamic atom motions in the protein were reduced.[10] This means that, as far as the thermostability is concerned, the hydrophobic solid surface is preferable since dynamic atom motions in luciferase are smaller on such a surface. Therefore, the solid surface should be prepared delicately to achieve a good balance between hydrophobic and hydrophilic conditions both from the viewpoint of preventing denaturation caused by immobilization and improving thermostability.

5. Conclusions

We find that luciferase adsorbs directly on a hydrophobic Si substrate, and via water molecules on a hydrophilic one. The adsorption-induced changes in conformation of luciferase are smaller on the hydrophilic Si substrate than on the hydrophobic one. The dynamic atom motions in luciferase are larger on the hydrophilic Si substrate than on the hydrophobic one. The adsorption-induced changes in the distances between the atoms responsible for holding luciferin are smaller on the hydrophilic Si substrate than on the hydrophobic one. In order to prevent the denaturation of luciferase caused by immobilization, the solid surface should be hydrophilic. For higher thermostability, however, the hydrophobic solid surface is preferable, since the dynamic atom motions in luciferase are smaller on such a surface. The solid surface should be prepared delicately both from the viewpoint of preventing denaturation caused by immobilization and improving thermostability.

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