The main factor of the decrease in activity of luciferase on the Si surface

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Abstract

The interactions between the luciferase surface area around the active site and the Si surface were estimated by molecular dynamics simulations. The results show that the luciferase surface area around the active site is more unstable than the luciferase surface area with relatively high hydrophobicity when luciferase adsorbs on the Si surface. The main factor of the decrease in activity of luciferase on the Si surface would be adsorption-induced structural changes in active site. Si surface should be designed focusing on structural changes in active site.

Key words: luciferase, Si surface, nanostructure, molecular dynamics simulation, biomedical application, protein chip

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1 Introduction

The inactivation of enzymes immobilized on solid surfaces is a serious problem for biomedical applications such as biosensors[1], bioreactor[2], because, the sensitivity of biosensor and the reaction efficiency of bioreactor are dependent on the intensity of enzymatic activities. In some reports, enzymes were linked on solid surfaces via the organic molecules in order to avoid inactivation[3,4]. These methods are available if these organic molecules do not inhibit the enzymatic activities.

Current Si-process technology enable us to fabricate a nanostructure array on Si surface[5,6]. Delicate control of the surface conditions, such as hydrophobic or hydrophilic properties, is also possible due to the recent progress of wet treatment technology for Si. Therefore a Si surface with a nanofabricated and wet-treated array may achieve compatibility with many kinds of enzymes.

As clues to understanding inactivation caused by immobilization, two inactivation mechanisms are suggested[3,4,7–9]. One is that the active site of enzyme is covered by solid surface, the other is structural change in active site of enzyme caused by impact of immobilization. If the main factor of the inactivation is identified, performance of biomedical applications is significantly improved.

The purpose of our study is to clarify the inactivation mechanism of an enzyme on an artificial surface and to suggest surface conditions suitable for immobilization. In our previous work, we analyzed the interactions between the

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luciferase_{hydrophobicity} (luciferase surface area with relatively high hydrophobicity) and the Si surface by molecular dynamics (MD) simulations[10]. In these simulations, firefly luciferase (EC 1. 13. 12. 7) was used. The result showed that the luciferase_{hydrophobicity} adsorbs on the Si surface. From the viewpoint of the orientation of the luciferase on the Si surface, the association of the substrate to luciferase is possible since the active site of luciferase is not covered by the Si surface in this adsorption state. In this work, we analyzed the interactions between the luciferase_{activesite} (luciferase surface area around the active site) and Si surface using MD simulations. As the Si surfaces, we have prepared a hydrogen-terminated Si surface as an example of a hydrophobic Si surface and a hydroxy-terminated Si surface as a hydrophilic Si surface.

Here we report the interactions between the luciferase_{activesite} and the Si surface, and the main factor of the decrease in activity of luciferase on the Si surface.

2 Methods

The MD simulations on the interactions between the luciferase_{activesite} and the Si surface were performed using the program AMBER 8.0[11]. The force fields in parm99[12] were used for these MD simulations. Structural data for luciferase were derived from the Protein Data Bank (PDB) (PDB code: 1ba3). The hydrogen-terminated Si surface is 0.5 nm thick in the [0 0 1] direction and 5.7 nm wide and long in [1 -1 0] and [1 1 0][10]. The hydroxy-terminated Si surface is 0.7 nm thick in [0 0 1] and 5.7 nm in [1 -1 0] and [1 1 0] [10]. During the simulations, atomic configurations of these Si surfaces were fixed. The partial atomic charges of the Si surfaces were determined on the basis

of Hartree-Fock molecular orbital calculations on a small cluster model[10]. In the initial states, a luciferase_{activesite} was placed facing the Si surface. Fig. 1 shows the luciferase_{activesite}. In the initial state, the distances between the residues (ARG218, LEU342 and LYS529) and the hydrophobic Si surface were 3.40 nm for ARG218, 3.33 nm for LEU342 and 2.48 nm for LYS529. In the initial state, the distances between the residues (ARG218, LEU342 and LYS529) and the hydrophilic Si surface were 3.33 nm for ARG218, 3.02 nm for LEU342 and 2.43 nm for LYS529. The luciferase_{activesite} - hydrophobic Si surface system was solvated with 15510 TIP3P water molecules[13] in a cubic box. The luciferase_{activesite} - hydrophilic Si surface system was solvated with 15460 water molecules in a cubic box. The procedure of this simulation was the same as that used to analyze the luciferase_{hydrophobicity} - Si surface system [10]. First, the initial structures were optimized by potential energy minimizations. Next, MD simulations were performed from the minimized structures. The temperature of the systems was gradually increased from 5 to 300 K in 52 ps and then kept at 300 K. Adsorption energies between luciferase and Si surfaces were estimated with MM-PBSA modules[14] in AMBER 8.0. The structural changes in luciferase were estimated with the analysis tools in GROMACS 3.3[15–19].

Fig. 1. Luciferase active site (highlighted).

3 Results and Discussion

First we performed the MD simulation of the luciferase_{activesite} - hydrophobic Si surface system. The simulation was performed for 2000 ps. The potential energies decreased within 1500 ps from the beginning of the simulation, and then converged. Hence we defined the time-averaged structure over the time after 1500 ps to be a stable structure. In the stable structure, the distances between the residues (ARG218, LEU342 and LYS529) and the hydrophobic Si surface were 3.20 nm for ARG218, 3.11 nm for LEU342 and 2.26 nm for LYS529. From the reduction in these distances in the stable structure, we find that the luciferase_{activesite} spontaneously comes close to the hydrophobic Si surface. Fig. 2 shows the initial and stable structure. From Fig. 2, we find that a few regions of the luciferase_{activesite} adsorbs on the hydrophobic Si surface. The adsorption energy of the luciferase_{activesite} was estimated to be -27.6 kcal/mol for the hydrophobic Si surface. The root-mean-square deviation (RMSD) value of luciferase_{hydrophobicstructure} (the stable structure of luciferase adsorbed on the hydrophobic Si surface) from luciferase_{isolatedstructure} (the stable structure of luciferase isolated in water) was estimated. The RMSD value of the whole parts was 0.23 nm. For the whole parts of luciferase_{isolatedstructure}, helix was 30.4 % and Sheet was 22.3 %. For the whole parts of luciferase_{hydrophobicstructure}, helix was 28.3 % and Sheet was 21.7 %. For the whole parts of luciferase_{isolatedstructure}, the radius of gyration was 2.49 nm. For the whole parts of luciferase_{hydrophobicstructure}, the radius of gyration was 2.50 nm. ARG218, PHE247 and LYS529 form hydrogen bonds to substrate luciferin [20]. For the luciferase_{isolatedstructure}, the distance between ARG218 and PHE247 was 1.05 nm, the distance between ARG218 and LYS529 was 2.11 nm and the distance between PHE247 and

LYS529 was 1.30 nm. For the luciferase_{hydrophobicstructure}, the distance between ARG218 and PHE247 was 0.99 nm, the distance between ARG218 and LYS529 was 2.11 nm and the distance between PHE247 and LYS529 was 1.29 nm.

Fig. 2. The initial and stable structure of the luciferase_{actsite} - hydrophobic Si surface system.

Next we performed the MD simulation of the luciferase_{actsite} - hydrophilic Si surface system. The simulation was performed for 2000 ps. The potential energies decreased within 1500 ps from the beginning of the simulation, and then converged. Hence we defined the time-averaged structure over the time after 1500 ps to be a stable structure. In the stable structure, the distances between the residues (ARG218, LEU342 and LYS529) and the hydrophilic Si surface were 3.25 nm for ARG218, 3.17 nm for LEU342 and 2.57 nm for LYS529. On the one hand ARG 218 comes close to the hydrophilic Si surface, on the other LEU342 and LYS529 back away from the hydrophilic Si surface. Fig. 3 shows the initial and stable structure. From Fig. 3, we find that a few regions of the luciferase_{actsite} adsorbs on the hydrophilic Si surface. The adsorption energy of the luciferase_{actsite} was estimated to be -6.3 kcal/mol for the hydrophilic Si surface. The RMSD value of luciferase_{hydrophilicstructure} (the stable structure of luciferase adsorbed on the hydrophilic Si surface) from luciferase_{isolatedstructure} was estimated. The RMSD value of the whole parts was

0.21 nm. For the whole parts of *luciferase_{hydrophilicstructure}*, helix was 29.6 % and Sheet was 22.1 %. For the whole parts of *luciferase_{hydrophilicstructure}*, the radius of gyration was 2.52 nm. For the *luciferase_{hydrophilicstructure}*, the distance between ARG218 and PHE247 was 0.97 nm, the distance between ARG218 and LYS529 was 2.34 nm and the distance between PHE247 and LYS529 was 1.55 nm.

Fig. 3. The initial and stable structure of the *luciferase_{activesite}* - hydrophilic Si surface system.

A few regions of the *luciferase_{activesite}* adsorbed on the hydrophobic Si surface, although the most regions of the *luciferase_{hydrophobicity}* adsorbed on the hydrophobic Si surface[10]. The adsorption energy of the *luciferase_{activesite}* was estimated to be -27.6 kcal/mol for the hydrophobic Si surface, although the adsorption energies of the *luciferase_{hydrophobicity}* was estimated to be -61.1 kcal/mol for the hydrophobic Si surface[10]. These results show that the *luciferase_{activesite}* is more unstable than the *luciferase_{hydrophobicity}* when *luciferase* adsorbs on the hydrophobic Si surface. A few regions of the *luciferase_{activesite}* adsorbed on the hydrophilic Si surface, although the partial regions of the *luciferase_{hydrophobicity}* adsorbed on the hydrophilic Si surface[10]. The adsorption energy of the *luciferase_{activesite}* was estimated to be -6.3 kcal/mol for the hydrophilic Si surface, although the adsorption energy of the *luciferase_{hydrophobicity}*

was estimated to be -32.3 kcal/mol for the hydrophilic Si surface[10]. These results show that the luciferase_{activesite} is more unstable than the luciferase_{hydrophobicity} when luciferase adsorbs on the hydrophilic Si surface. Chances are small that the active site of luciferase is covered by the Si surface both on the hydrophobic Si surface and on the hydrophilic Si surface. Therefore the main factor of the decrease in activity of luciferase on the Si surface would be adsorption-induced structural changes in active site of luciferase. The structural changes in active site residues of luciferase were smaller on the hydrophobic Si surface than on the hydrophilic Si surface, although the structural changes in the whole parts of luciferase were smaller on the hydrophilic Si surface than on the hydrophobic Si surface when the luciferase_{activesite} adsorbed on the Si surface. The structural changes in active site residues of luciferase were atypical. The nanofabrication and wet-treatment techniques reduce adsorption-induced structural changes in active site of luciferase on the Si surface[21]. In order to improve performance of biomedical applications, Si surface should be designed focusing on structural changes in active site.

4 Conclusions

We analyzed the interactions between the luciferase surface area around the active site and the Si surface. The luciferase surface area around the active site is more unstable than the luciferase surface area with relatively high hydrophobicity when luciferase adsorbs on the Si surface. Chances are small that the active site of luciferase is covered by the Si surface. The main factor of the decrease in activity of luciferase on the Si surface would be adsorption-induced structural changes in active site of luciferase. Si surface should be designed

focusing on adsorption-induced structural changes in active site.

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* Graphical Abstract (synopsis)

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