Effect of double-stranded DNA on electrical double layer structure at oxide/electrolyte interface in classical molecular dynamics simulation

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The elucidation of the electrical double layer (EDL) structure including biomolecules is helpful in controlling biointerfacial functions in biosensing devices. In this study, the effect of double-stranded DNA (ds-DNA) on the EDL structure at the SiO₂/NaClaq interface in a concentrated solution was investigated by classical molecular dynamics (MD) simulation. In 1.0 M NaClaq, negatively charged ds-DNA shielded electrically by cations did not affect the potential profile of the EDL structure. This finding indicates that the simulated bio-interfacial structure allows the detection of ionic charges adsorbed directly onto substrates in a concentrated solution regardless of the presence of shielded biomolecular charges. (100 words)

1. Introduction

Most biosensors enable the detection of bio-molecular recognition events at a substrate/solution interface. The elucidation of the interfacial behavior is helpful in controlling the detection sensitivity and selectivity of biosensors. In particular, semiconductor-based field-effect transistors (FETs) [1] are available for the detection of bio-molecular charges or ionic behaviors based on various

biological phenomena. A FET biosensor is based on the potentiometric detection of biomolecular charges at an oxide membrane as a sensing surface, where ions are adsorbed specifically or the specific binding of biomolecules is induced. Therefore, the principle of the FET biosensor enables the direct detection of various biological phenomena in a label-free manner based on the intrinsic ionic or biomolecular charges, as reported in previous papers [2-11]. Thus, FET biosensors are useful for simple and cost-effective systems in the field of clinical diagnosis and pharmaceutical discovery.

However, the electrical behaviors of ions or bio-molecules at the substrate/solution interface of a FET biosensor have not yet been clarified in detail. The Debye length, which is based on the deviation of ionic charges localized at the sensor surface, is critical to the evaluation of the interfacial structure of FET biosensors [7,12,13], but it is difficult to clarify simultaneously and experimentally the dynamics of components such as ions, water molecules and bio-molecules at a charged oxide surface in a solution. As an example of such a study, the effect of the length of extended DNA molecules on electrical signals was investigated using a FET biosensor in a previous work, which showed that the FET biosensor was insensitive to DNA molecules longer than 41 bases because the DNA molecular charges far from the sensing surface were shielded by the ionic charges in the bulk solution [7]. From this finding, it was assumed that the FET biosensor could not detect molecular charges in the bulk solution beyond the Debye length, but this assumption has not been explained considering any elements around the solid/liquid interface. In addition, the respiration activity of living cells can be detected easily as the change in interfacial pH between a cell and the sensor surface on the basis of the principle of the FET. However, this finding seems inconsistent with the fact that the bulk solution would have approached to near the sensing surface, that is, the Debye

length might have been extremely small owing to the high ion strength in the cell culture medium.

This assumption also has not been examined in detail and cannot be clarified experimentally.

On the other hand, a theoretical procedure is helpful for understanding ionic behaviors at the substrate/solution interface with biomolecules. The possibilities of selective detection of the antigen-antibody reaction [12] and DNA hybridization [13] were indicated theoretically. These studies provided the fundamental concepts of the interfacial characteristics in the sensing of environments; however, the extent to which theoretical calculations realistically reflect actual phenomena including the dynamics of ambient water molecules and other ions remains a problem. On the other hand, some classical molecular dynamics (MD) simulations with the DNA tethered onto the solid state have been carried out [14-21]. They contributed to the comprehension of the dynamics of tethered DNA, but the ionic or water molecular behavior at the solid/liquid interface has not been considered. Therefore, we have clarified the fundamental properties of the electrical double layer (EDL) structure in a concentrated solution without biomolecules [22,23]. To further clarify such properties, in this study we investigated the effect of biomolecules on the ionic behaviors at the device/solution interface by use of the MD simulation, resulting in the development of a pseudo-experimental system of FET biosensors. In particular, we simulated the EDL structure with the tethered DNA in a concentrated solution and compared it with that without such a biomolecule.

2. Methods

2.1 Simulation cells

To compare the effect of the biomolecules on the aqueous structure around the solid/liquid

interface, we prepared simple oxide/electrolyte interfacial simulation cells without bio-molecules (solid/liquid (SL) model) and simulation cells with double-stranded DNA immobilized on the oxide surface (SL/DNA model). (100) SiO₂ (alpha-quartz) was chosen as the oxide solid surface. SiO₂ is widely used as an insulator in FET devices. The SiO₂ surface was protonated in a solution depending on the pH, and adjusted to a ratio of $N_{\text{SiO}}/N_{\text{SiOH}} = 1/5$ in the simulations [22,23]. The electrolytes used were prepared as 1.0 M NaCl solutions in both simulations. In the case of the simulation cells of the SL/DNA model, 19 Na⁺ atoms were inserted additionally to neutralize the DNA molecular charges. The initial DNA strand was the B chain consisting of the dodecamer d(AAAAAAAAAA) and the complementary strand of base-T. This ds-DNA structure was generated from the nucgen module of AMBER10 [24]. The ds-DNA probe was immobilized on the SiO₂ layer using the DNA linker, which was generated from propyl glycidyl ether and 1-aminohexane by the ring-opening addition reaction [14]. The propyl glycidyl ether and SiO₂ were chemically bonded by silane coupling treatment and the terminus of 1-aminohexane was connected to the phosphate of the 5'-end of the DNA duplex. The simulation cell contained 6360 H₂O, 120 Na, and 120 Cl atoms in the SL model, and 6360 H₂O, 139 Na, and 120 Cl atoms in the SL/DNA model, totally summed up to 23087 and 23782 atoms, respectively. The periodic boundary condition was applied to rectangular cells of $54.052 \times 49.130 \times z \text{ \AA}^3$ ($z = 111.44 \text{ \AA}$ in the SL model and $118.35 \text{ \AA}$ in the SL/DNA model) in all directions. Then, the NaCl solution was placed above the SiO₂ substrate, and a vacuum region of 20 Å was inserted at the top of the cell to relax the pressure in the solution and suppress the interaction over the periodic boundary. The atoms in the bottom layer of the SiO₂ were immobilized during the calculations. The chemical structure of the SL/DNA model is shown in Fig. 1a.

2.2 Molecular dynamics simulation

Geometric optimization was carried out for each cell before the main MD calculations by a complex method of calculation involving the steepest descent and conjugate gradient methods with a total of 5000 steps. Then, both simulation cells were relaxed by MD in the NVT ensemble from 100 K to 300 K with a 100 K increment for 0.5 ns after geometric optimization. The main MD calculations were performed at 300 K for 3.0 ns in the NVT ensemble. The temperature was controlled using a Nose thermostat [25]. The Newton equation was solved using the velocity Verlet algorithm [26] with a time step of 1.0 fs. The cutoff distance for short-range interactions was 12.5 Å, whereas the Coulomb interactions were calculated by the Ewald method [27]. The last 0.5 ns was used for various analyses. All simulations were carried out using the Materials Studio simulation package [28] including Condensed Phase Optimized Molecular Potentials for Atomic Simulation Studies (COMPASS) [29,30]. Almost all charge parameters were assigned by COMPASS, but the charges of the phosphate of the ds-DNA were modified to satisfy the chemical requirements. The ds-DNA charge parameters are shown in Fig. 1b.

3. Results and Discussion

3.1. Configuration of water molecules and ions in SL/DNA model

Figure 2 shows the snapshots of the tethered DNA in the SL/DNA model and the SL model for the temporal changes of the dynamics. The snapshots indicated that Na⁺ accumulated at the interface in both models. One base at the 3'-end of the polyT strand dissociated from the complementary polyA strand; however, the double-stranded structure remained for 3.0 ns. The terminus of ds-DNA

could hardly maintain the double strand structure because the dissociated base was nearest to the negatively charged SiO_2 surface. The steric constraint and electrical interaction prevented the base pair from maintaining the double strand structure in addition to their small stacking at the DNA terminus. Although the ds-DNA was rigid owing to its helix structure, the DNA linker oscillated in the solution because of the carbon chain, resulting in the leaning of the tethered DNA. In this simulation, the ds-DNA drifted in the range from 25 Å to 60 Å in the z-direction in 3.0 ns.

Focusing on the aqueous structures at the oxide surface, the axial distribution function (ADF) along the z-direction in the SL/DNA model is compared with that in the SL model, as shown in Fig. 3. The distribution of the hydrogen and oxygen atoms of water molecules and that of Na^+ and Cl^- are shown in Figs. 3a and 3b, respectively. Their distributions in the SL model can also be found in previous papers [22,23]. In both models, the water molecules near the interface formed a three- or four-layer structure on the SiO_2 surface and the hydrogen peaks were not consistent with the oxygen peaks owing to the structure of water molecules. The charged solid state and the adsorbed ions induced the dipole orientation of water molecules; consequently, the hydrogen-rich region or oxygen-rich region appeared separately at the interface. This inconsistency between the hydrogen and oxygen peaks in the ADF vanished in the bulk solution approximately 10 Å further from the surface. On the other hand, the adsorbed Na^+ showed two sharp peaks in both models. Na^+ ions occupied the area around each peak position owing to the ionic hydration depending on the layered structure of water molecules. Firstly, Na^+ ions were interacted with the water layer from the bulk solution (the outer peak); subsequently, they moved closer to the surface because of their higher concentration (the inner peak), as reported in a previous work [23]. As a result, Na^+ exhibited two

peaks in the vicinity of the SiO₂ substrate. These adsorbed Na⁺ ions accumulated at >SiO⁻ species and formed a bond similar to that in >SiO-Na, whereas the distribution of Cl⁻ was found to be above the Na⁺ peaks along the z-direction. Comparing the ADFs in the SL/DNA model with those in the SL model for the hydrogen and oxygen distributions, negligible differences were observed from 15 to 30 Å. Moreover, no changes in the Na⁺ and Cl⁻ distributions were found, regardless of the inclusion of DNA molecules in the simulated models, except for the Na⁺ peak nearest the surface becoming slightly more intense in this model because the SL/DNA model included 19 additional Na⁺ atoms and the Na⁺ concentration of the SL/DNA model was slightly higher. Therefore, it was concluded that the tethered ds-DNA at the SiO₂ surface did not affect the interfacial aqueous structure in the concentrated 1.0 M NaCl solution.

3.2. Charge and potential profiles in SL/DNA model

Fig. 4a shows the charge distribution for all charged atoms along the z-direction in both models. In both models, the charge profiles consisted of one large peak and three small peaks for the solution near the interface and remained neutral in the bulk region. The first large positive peak originated from the positive charges of Na⁺ and the hydrogen of water molecules, and the second small positive peak mainly originated from the adsorbed Na⁺, which was sufficient to cancel the negative charge from the oxygen of the water molecules. The subsequent negative peaks were due to Cl⁻ interacting with Na⁺, because Na⁺ and Cl⁻ do not completely dissociate in a concentrated solution [22,23,31]. It was not surprising that no significant difference was found between the two interfacial charge profiles, because the ADFs in each model were very similar. However, the charge profile in the SL/DNA model also remained zero in the bulk solution despite the existence of both ends of

ds-DNA in the range of 40 to 60 Å. This is because more Na⁺ ions were introduced in the SL/DNA model than in the SL model, and the ds-DNA was surrounded by Na⁺ ions, which shielded the negative charges of the phosphate ester of the nucleotides, as will be described later. In addition, no deviation of the amounts for hydrogen and oxygen of water molecules was found in this region.

The potential profile along the z-direction obtained by solving the Poisson equation under the Neumann boundary condition is shown in Fig. 4b. We defined the reference point at 40 Å as the middle of the solution box and recalculated to set the potential to zero. Potential profiles were classified to three domains, as reported previously [22,23], which corresponded to the linear drop, linear rise, and gradually decreasing sections. The potential profile in the SL/DNA model was reasonably consistent with that in the SL model and the surface potential did not change regardless of whether the ds-DNA was tethered or not. Therefore, it is concluded that DNA recognition events such as hybridization and extension can not be detected using FET biosensors in a concentrated solution. This inalterability of the potential profiles indicated that ds-DNA does not affect the EDL structure and surface potential. Expanding this hypothesis, the charged biomolecules drifting near the interface do not affect the surface potential in a concentrated solution. Thus, this finding indicates the possibility that the concentration change of various ions or pH can be detected selectively on the basis of their interaction with >Si-O⁻/OH/OH₂⁺ species without being interrupted by charged biomolecules under physiological conditions.

In Fig. 4, a gradient of the potential profile was found in the range of $z > 25$ Å, although the charge density was zero. In a dilute solution, counterions generally form a Helmholtz layer and diffusion layer on the basis of the Stern model. In this model, the charge of the solid surface is

completely shielded at the end of the diffusion layer, and the electrostatic potential is saturated and neutralized outside of the diffusion layer. On the other hand, we calculated the charge and potential profiles for the concentrated solution in this simulation, in which we set the negative charge at the SiO₂ substrate. As a result, we found that Na⁺ ions accumulated around the SiO₂ surface. However, the amount of adsorbed Na⁺ at the surface was not sufficient to cancel out the negative charge of the SiO₂ surface. This is because Na⁺ strongly interacted with Cl⁻, and they moved together in the vicinity of the surface depending on the ionic concentration [23]. Therefore, the gradient of the electrostatic potential appeared at $z > 25 \text{ \AA}$, even though the charge density was zero there. However, the potential will approach asymptotically toward zero in the bulk solution if the simulated cell size is so largely designed that the negative charges at the SiO₂ surface are canceled by the ionic behaviors in the bulk solution. In the simulation cell used in this study, the sufficient number of ions, water molecules and DNA molecule at the SiO₂ substrate was adequately prepared for the simulation, focusing on the DNA dynamics and the ionic behaviors in the vicinity of the solid/liquid interface, while the ionic behavior in the bulk solution was not of main interest.

3.3. Ion condensation around ds-DNA

Although the DNA molecule has negative charges derived from the phosphate ester, how the tethered DNA affects the aqueous structure has not yet been classified. Fig. 5a shows snapshots of the tethered DNA and Na⁺ in the space-filling model at 3.0 ns. The Na⁺ arranged along the ds-DNA chain and looked like a double-strand structure. Fig. 5b shows the radial distribution function (RDF) and the coordination number (CN) for the distance between the phosphorus at the side chain of ds-DNA and Na⁺. The first peak of the RDF at less than 3 Å showed a high intensity of $g(r) > 60$,

which indicated a strong interaction between atoms. Therefore, the tethered DNA was constantly surrounded by Na^+ ions despite the oscillation of the ds-DNA. On the other hand, the number of Na^+ ions within 4 Å from the phosphorus was approximately one. One Na^+ ion was electrically bonded to one phosphate ester to maintain electrical neutrality. Generally, Na^+ ions play an important role in maintaining the double strand structure. Indeed, if there are much fewer ions in the solution, the DNA cleaved immediately [32]. In this MD simulation, the adsorbed Na^+ prevented the ds-DNA from cleaving and shielded the negative charge of the phosphate group; thus, the aqueous structure around the interface was less affected by the tethered DNA.

4. Conclusions

In this study, we investigated the effect of the tethered ds-DNA at the SiO_2 surface on the aqueous EDL structure in 1.0 M NaCl solution. The tethered DNA maintained its double strand structure for 3.0 ns in this simulation. In the concentrated solution, the introduction of ds-DNA at the substrate did not contribute to the change in the atomic configuration at the $\text{SiO}_2/\text{NaCl}_{\text{aq}}$ interface. The charge and potential distributions in the SL/DNA model were reasonably consistent with those in the SL model in the bulk solution as well as near the interface. This correspondence was due to Na^+ adsorption onto ds-DNA, resulting in the neutralization of DNA charges.

In particular, the MD simulation demonstrated that the shielding of DNA molecular charges by cations should be considered as necessity for DNA molecular recognition in a concentrated solution using the FET biosensors with DNA probes. Moreover, the simulation technique has clarified that FET biosensors can detect the ionic charges adsorbed directly at the SiO_2 surface regardless of the

shielded charges of probe molecules in a concentrated solution. For the case of cellular analyses, the findings of this MD simulation indicate that the pH variation at the cell/substrate interface associated with the respiration activity of living cells can be detected even in a cell culture medium including various types of proteins or ionic polyelectrolytes using FET biosensors [11]. Even in a concentrated solution including polymer compounds, monovalent ions such as counterions may affect the electric double strand layer and potential distribution regardless of the type of compound. However, the elucidation of the EDL structure including bio-molecules in a dilute solution is required in order to detect the target biomolecules themselves in the future.

Acknowledgements

This work was partly supported by the CREST project, Japan Science and Technology Agency (JST). We thank Dr. Abhijit Chatterjee of Accelrys K.K., Japan, for help and useful discussion.

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FIGURE CAPTIONS

Fig. 1 (a) Chemical structure around biointerface utilized in MD simulations. The symbols A and T indicate adenine and thymine, respectively. (b) Charge parameters of ds-DNA. The charges of the phosphate were reconfigured as 1.019 eC for P and -0.776 eC for O. The total charge of the ds-DNA was adjusted to -19 eC.

Fig. 2 (a) Snapshots of ions and tethered DNA around SiO₂/NaClaq interface at 0 ns, 1.0 ns, 2.0 ns, and 3.0 ns. Water molecules are not shown in the snapshots, but they were included in this simulation. (b) Snapshots of ions and water molecules around SiO₂/NaClaq interface at the same times. In the NaCl solution, the purple and the light-green balls denote Na⁺ and Cl⁻, respectively.

Each coordinate axis is fixed, and the z-axis shows the direction perpendicular to the SiO₂ surface.

The SiO₂ substrate is located at the bottom of the snapshots.

Fig. 3 Configurations of (a) water molecules and (b) Na⁺ and Cl⁻ along the z-direction perpendicular to the SiO₂ interface in the solid/liquid/DNA model and the solid/liquid model. The ordinate indicates the ratio of the atomic density to the bulk density of each atom. The position of the SiO₂ surface that came in contact with NaCl_{aq} was set at about 17 Å in the simulation cells, as indicated by the dotted line.

Fig. 4 (a) Charge distribution for all atoms calculated from the atomic density profile in the solid/liquid/DNA model and the solid/liquid model. The charge distribution was calculated by accumulating all atomic charges including not only NaCl solution but also SiO₂. (b) Potential distribution along the z-direction obtained by solving the Poisson equation. The calculation was performed on the basis of the charge distribution shown in (a). The position of the SiO₂ surface that came in contact with NaCl_{aq} was set at about 17 Å in the simulation cells, as indicated by the dotted line.

Fig. 5 (a) Snapshots of the enlarged view of the tethered DNA and adsorbed Na⁺ at 3.0 ns. The purple balls representing Na⁺ are shown in the space-filling model. (b) Radial distribution function (RDF) and coordination number (CN) of Na⁺ for phosphorus of ds-DNA. The left ordinate represents the RDF (black line), and the right ordinate represents the CN (red line).

Figure 2

Figure 3

a.

b.

Figure 4

a.

b.

Figure 5

*Water molecules are not shown here
for clarity of image.

Graphic Abstract