ORIGINAL ARTICLE

Synthesis of novel nucleobase-terminated organosilane and its self-assembly on a substrate

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A novel nucleobase-terminated organosilane with nucleobase and ethoxysilane moieties was synthesized by a simple method in which bromouracil reacts with amino-terminated organosilane without solvents. The uracil-terminated organosilane was self-assembled on a hydroxylated surface, and then the uracil-concentrated surface was obtained. The resulting uracil-terminated self-assembled layers were characterized by X-ray photoelectron spectroscopy and ellipsometry. Adenine complementary to uracil was effectively adsorbed onto the resultant uracil-concentrated surface by multiple hydrogen bonds. Herein, a simple method is provided for synthesizing a nucleobase-terminated organosilane and modifying a hydroxylated surface to a nucleobase-concentrated surface. Our method, which uses the self-assembly of nucleobase-terminated organosilanes, facilitates the construction of bioconjugated devices for separating and sensing nucleobases and nucleic acids. *Polymer Journal* (2012) 44, 625–631; doi:10.1038/pj.2012.41; published online 28 March 2012

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INTRODUCTION

Self-assembled monolayers (SAMs) and multilayers are ultrathin films that are spontaneously formed by the assembly of active molecules on substrates.^{1–7} Self-assembled layer processes are inexpensive and versatile nanofabrication techniques that generate nanoscale structures and nanostructured materials for molecular electronics, optics, memory devices and sensors.^{8–12} In particular, self-assembled layers on sensor chips and nanoparticles have been incorporated into the construction of useful bioconjugated devices for various biomedical applications, such as sensing, bioimaging and drug delivery system (DDS).

Active molecules that form SAMs typically have a head group for attaching to a solid substrate and an alkyl chain tail with functional groups. The representative molecules for SAMs are alkanethiols and organosilanes, which are typically used on metals and hydroxylated substrates, respectively. In particular, various organosilanes have been utilized as coupling agents for surface modification to control adhesions and improve the miscibility of composites and hybrid materials.^{13–17} SAMs and multilayers of organosilanes on various substrates, such as films, particles and fibers, are also applicable to the development of biomaterials with surfaces that are well-designed for enhancing biocompatibility or sensing target biomolecules as markers.

Molecular recognition, such as the formation of antigen–antibody complexes and the complementary hydrogen bonds of nucleic acids, is an essential phenomenon of living systems. For example, in the double helix of DNA and RNA, a nucleobase on one strand precisely

interacts with its complementary nucleobase on the other strand; this interaction is referred to as base pairing. The complementary base pairing of DNA and RNA is the most important cellular molecular recognition event, which is based on the formation of two or three hydrogen bonds per nucleobase. DNA microarrays and chips in which probe DNAs are covalently attached to a solid substrate are widely used as diagnostic devices for measuring the expression levels of genes and detecting single-nucleotide polymorphisms. Various methods for organizing nucleic acids and nucleobases on a substrate have been undertaken to develop DNA chips and microarrays for genetic diagnosis. For example, thiol-modified DNA oligomers were covalently attached to SAMs formed from organosilanes with a functional group.^{18,19}

Standard methods for fabricating SAMs and multilayers of nucleic acids and nucleobases require several steps. First, an appropriate substrate is immersed into a solution containing active molecules with a head group for attaching to a substrate and a functional group for binding to nucleic acids and a nucleobase. After the active molecules are self-assembled on a substrate, nucleic acids and nucleobases are covalently bound to the functional groups of the active molecules on a substrate to form SAMs and multilayers of nucleic acids and nucleobases. Although SAMs and multilayers of nucleic acids and nucleobases can be fabricated by these simple steps, these processes have some disadvantages, such as the undesirable residues of the functional groups of the active molecules and complicated processes, including inefficient reactions. However, the synthesis of an active

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molecule with a head group and a nucleobase moiety enables nucleobase self-assembled layers to be fabricated in an one-step process, which is the most simple and versatile method.

The synthesis of nucleobase-terminated organosilane with both nucleobase and silane moieties is difficult because nucleobases are insoluble in most solvents and silanes are easily condensed in the presence of a small amount of water. However, we successfully synthesized a novel nucleobase derivative that is self-assembled on a hydroxylated surface using a simple method, and we describe a simple method for synthesizing nucleobase-terminated organosilane without solvents. The self-assembly of the nucleobase-terminated organosilane on a hydroxylated surface and the adsorption of a complementary nucleobase onto the resultant nucleobase-concentrated surface were investigated.

EXPERIMENTAL PROCEDURE

Materials

For this study, 3-(aminopropyl)triethoxysilane (APTES) and 5-bromouracil were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan) and used as received. Adenine was purchased from Sigma-Aldrich (St Louis, MO, USA) and used as received. Cyclohexanone was purchased from WAKO Pure Chemical Industries (Osaka, Japan) and used as received. All of the other solvents and reagents were of the analytical grade; they were obtained from commercial sources and used without further purification.

Synthesis of 5-(3-(triethoxysilyl)propylamino)pyrimidine-2,4(1H,3H)-dione

The uracil-terminated organosilane (5-(3-(triethoxysilyl)propylamino)pyrimidine-2,4(1H,3H)-dione (Ura-TES)) was synthesized by the reaction of 5-bromouracil with APTES without solvents as follows (Scheme 1): 5-bromouracil (10.5 mmol) was added to APTES (67.8 mmol), and the suspension was stirred at 60 °C for 10 h under reduced pressure. The produced precipitation was filtered and washed with ethanol. The final product was dried under a vacuum to obtain Ura-TES as a white solid with a 38% yield. The resultant Ura-TES was identified by nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy measurements. ¹H-NMR (400 MHz, CDCl₃) δ p.p.m.: 6.45 (s, 1H, =CHNH-), 3.93 (t, *J* = 5.8 Hz, 1H, -NHCH₂-), 3.82 (q, *J* = 7.0 Hz, 6H, -OCH₂CH₃), 2.91 (q, *J* = 6.6 Hz, 2H, -NHCH₂CH₂-), 1.72 (quin, *J* = 7.8 Hz, 2H, -CH₂CH₂Si-), 1.22 (t, *J* = 7.1 Hz, 9H, -OCH₂CH₃), 0.66–0.70 (m, 2H, -CH₂Si-). ¹³C-NMR (100 MHz, CDCl₃) δ p.p.m.: 161.3, 151.3, 125.5, 111.5, 58.4, 46.5, 22.1, 18.3 and 7.8.

Self-assembly of Ura-TES on a substrate

Regular (100) silicon wafers as substrates were cleaned by being soaked in concentrated sulfuric acid and washed with deionized water. The cleaned silicon substrates were immersed in a cyclohexanone solution of 2 mM Ura-TES at 70 °C for time periods ranging from 1 to 48 h. After the reaction, the substrates were rinsed with chloroform and dried under a vacuum. The amount of Ura-TES attached to the silicon substrate was measured as follows. The Ura-TES self-assembled substrates were immersed in 1 M HCl solution at 100 °C for 1 h to hydrolyze the binding between Ura-TES and the substrate. The amount of Ura-TES detached by the hydrolysis was determined colorimetrically from the absorbance at 280 nm using the UV-2500PC spectrophotometer (Shimadzu Co., Kyoto, Japan).

Scheme 1 Synthesis of uracil-terminated triethoxysilane (Ura-TES).

Characterizations of Ura-TES self-assembled layers on a substrate

The surface structure of the Ura-TES self-assembled layers on the silicon substrate was observed with atomic force microscopy (AFM) in the tapping mode using SPI3800/SPA400 (SII nanotechnology, Tokyo, Japan), taking 256 points in a 1000 × 1000 nm area with a 0.5 Hz scan rate at room temperature. From these AFM measurements, a roughness analysis was also performed on the uracil-immobilized substrates according to the manufacturer's software program. The root mean square surface roughness was calculated as the s.d. of all of the height values within the given area. The chemical structure of the Ura-TES self-assembled layers on a substrate was characterized with X-ray photoelectron spectroscopy (XPS) using the ESCA-3400 (Shimadzu Co.). An Mg Kα X-ray source was used at a power of 200 W (20 mA × 10 kV), and the pass energy was set at 75 eV. The pressure in the analysis chamber was ~4.0 × 10⁻⁷ Pa. The charge correction in the binding energy scale was performed by setting the -CH₂- peak in the carbon spectra to 285.0 eV. Overlapping peaks were resolved into their individual core-level spectra components, which were well described by the Gaussian distributions. The full width at half maximum of each individual peak was <2.0 eV. The thickness of the Ura-TES self-assembled layers on the substrate was determined with an imaging ellipsometer (Nippon Laser & Electronics Lab., Nagoya, Japan) equipped with a He-Ne laser (632.8 nm, 10 mW). The refractive index of the organosilane layer was assumed to be approximately equal to that of silica. (*n* = 1.465).²⁰

Adsorption of adenine on Ura-TES self-assembled layers

The uracil-immobilized substrates on which Ura-TES was self-assembled under various conditions were immersed in an aqueous solution of 10 mM adenine adjusted to a pH of 2.0 using HCl at 25 °C for time periods ranging from 1 to 48 h. The amount of adenine adsorbed onto the uracil-immobilized substrates was determined by measuring the amount of adenine in the supernatant from the absorbance at 280 nm using the UV-2500PC spectrophotometer (Shimadzu Co.). XPS measurements were also conducted to confirm the adsorption of adenine onto the uracil-immobilized substrate. The atomic ratios (O_{1s}/N_{1s}) of the uracil-immobilized and adenine-adsorbed substrates were determined by considering the integrated peak areas of N_{1s} and O_{1s} and their respective experimental sensitivity factors. Experimental sensitivity factors of 0.505 and 0.736 were used for N_{1s} and O_{1s}, respectively.

RESULTS AND DISCUSSION

Synthesis of nucleobase-terminated organosilane

Organosilanes are covalently attached to a wide variety of hydroxylated surfaces, including glass, silica particles and various polymers with hydroxyl groups.²¹ A variety of organosilanes have been utilized as silane coupling agents to improve the compatibility between inorganic fillers and a polymer matrix when preparing polymer-based reinforced composites. The self-assembly of organosilanes with a functional group has also attracted considerable attention in the surface functionalization of films, particles and fibers. Biomolecules such as proteins, saccharides and DNAs are covalently attached to surfaces modified by organosilanes with various functional groups. Thus, surface functionalizations by the self-assembly of organosilanes have been used to construct bioconjugated devices, such as biosensors and bioseparators.^{18,19,22,23} In the standard methods for preparing bioconjugated devices based on the self-assembly of organosilanes, after organosilanes with a functional group such as carboxyl and amino groups are self-assembled on hydroxylated surfaces, biomolecules are covalently attached to the functional groups on the modified surfaces. Thus, in these methods, the preparation of biomolecule-attached surfaces requires several processes. When the reaction of biomolecules with functional groups on a substrate surface is not efficient, residual functional groups on the surface often affect the properties and functions of the biomolecule-immobilized devices. In contrast, biomolecule-terminated organosilanes are highly promising bioconjugated molecules for constructing biomolecule-

attached surfaces because their self-assembly enables biomolecules to be attached to a substrate surface by an one-step process. In this study, we tried to synthesize a uracil-terminated organosilane by the reaction of bromouracil with APTES in solvents. However, there is no co-solvent in which both bromouracil and APTES are soluble. Furthermore, APTES easily undergoes a condensation reaction in the presence of a small amount of water. Therefore, it is difficult to directly produce the uracil-terminated organosilane by the reaction of bromouracil with APTES. From the investigations of the synthetic process, we found a simple method for easily synthesizing the uracil-terminated organosilane with both uracil and ethoxysilane moieties. Ura-TES was very easily synthesized as the uracil-terminated organosilane by the reaction of bromouracil with APTES without solvents.

Bromouracil is insoluble in APTES at room temperature. When the suspension in which bromouracil was dispersed in APTES was heated with stirring at 60 °C, a precipitation of bromouracil disappeared gradually, and the solution became transparent. After a few hours, the product began to precipitate as a white solid. The obtained product was purified by being filtered and washed with ethanol. The final product was dried under a vacuum to obtain Ura-TES as a white solid with a 38% yield. The $^1\text{H-NMR}$ spectra of the purified Ura-TES, bromouracil and APTES are shown in Figure 1. In the spectrum of Ura-TES shown in Figure 1a, the signals assigned to the uracil and ethoxysilane moieties are confirmed. It should be noted that the ethoxysilane moieties underwent no condensation during the reaction of bromouracil with APTES. Figure 1 demonstrates that a uracil-terminated organosilane can be synthesized by the simple reaction of bromouracil with APTES without solvents. Thus, consisting of a one-step reaction without solvents, our simple method for synthesizing nucleobase-terminated organosilanes is very convenient. The resultant organosilane is a unique molecule with a nucleobase moiety as a

molecular recognition site and a silane moiety for attaching to hydroxylated surfaces; the molecule is applicable to the fabrication of nucleobase-attached surfaces by its self-assembly on hydroxylated surfaces.

Self-assembly of Ura-TES on a substrate

SAMs and multilayers are ordered molecular assemblies formed by the chemisorption of active molecules onto a solid surface. Alkanethiolates and organosilanes are representatives of active molecules that form SAMs on gold and hydroxylated surfaces, respectively. In particular, organosilanes are widely utilized as silane coupling agents to chemically modify solid surfaces. The utilization of organosilanes with a functional group enables hydroxylated surfaces to be chemically modified into functional group-concentrated surfaces.^{2,15} Therefore, nucleobase-terminated organosilane is an active molecule used to fabricate nucleobase-concentrated surfaces by its self-assembly on a substrate. In this study, we investigated the self-assembly of Ura-TES on a silicon wafer as a solid substrate. A small amount of water in the solvent results in the predominant condensation of Ura-TES in the solution phase rather than the attachment reaction of Ura-TES with hydroxylated surfaces.²⁴ Owing to the predominant condensation of Ura-TES in the presence of a small amount of water in the solvent causes the formation of its aggregates in the solution phase, it is difficult to form homogeneous Ura-TES self-assembled layers on a substrate. Owing to its relatively high boiling point and immiscibility in water, cyclohexanone is a good solvent for Ura-TES. Thus, this study used cyclohexanone as a solvent for the self-assembly of Ura-TES. After Ura-TES was dissolved in cyclohexanone, a silicon wafer was immersed in the solution as a solid substrate for the reaction with the ethoxysilane groups of Ura-TES.

Figure 1 $^1\text{H-NMR}$ spectra of (a) Ura-TES, (b) bromouracil and (c) APTES.

Figure 2 shows the AFM images of the Ura-TES self-assembled layers on a silicon substrate after the substrate was immersed in a cyclohexanone solution of Ura-TES for time periods ranging from 1 to 48 h. Although a surface of the original silicon substrate was flat, the surface of the substrates immersed in a cyclohexanone solution of Ura-TES became rough. Furthermore, the surface roughness of the Ura-TES self-assembled layers was estimated from the AFM images by measuring the root mean square surface roughness. The root mean square values increased slightly as the immersion time increased

(Supporting Information), indicating that Ura-TES did not form a monolayer; instead, it formed a multilayer on the silicon substrate. Thus, the AFM observations demonstrate that the self-assembly of Ura-TES occurred on the substrate surface, and the amount of self-assembled Ura-TES increased as the immersion time increased. It should be noted that no large Ura-TES aggregates were observed on the substrate surface, indicating that Ura-TES was homogeneously self-assembled on the substrate surface without the formation of aggregates by self-condensation in the solution phase. The order of

Figure 2 Topographical AFM images ($1 \times 1 \mu\text{m}$) of Ura-TES self-assembled layers on the substrate when the substrate was immersed in a cyclohexanone solution of 2 mM Ura-TES: (a) original silicon substrate and Ura-TES self-assembled layer after immersion for (b) 1 h, (c) 2 h, (d) 4 h, (e) 8 h, (f) 15 h, (g) 24 h, (h) 36 h and (i) 48 h. A full color version of this figure is available at *Polymer Journal* online.

Table 1 Experimental and theoretical area ratios of $\text{C}_{1\text{s}}$ component peak of (5-(3-(triethoxysilyl)propylamino)pyrimidine-2,4(1H,3H)-dione (Ura-TES) self-assembled layer on a substrate

	Structure	Peak ^a	Position (eV)	Width (eV)	Area ratio (%)
Experimental	Ura-TES self-assembly	1	285.1	1.53	72.3
		2	286.6	1.02	7.2
		3	288.2	2.74	20.5
Theoretical		1	285.1	—	61.5
		2	286.6	—	23.1
		3	288.2	—	15.4
	1	285.1	—	71.4
		2	286.6	—	0
		3	288.2	—	28.6

^aPeak 1 is assigned to $-\text{CH}_2-$ and $-\text{C}-\text{NH}-$, Peak 2 is assigned to $-\text{C}-\text{O}-$ and Peak 3 is assigned to $>\text{C}=\text{O}$.

reactivity of the organosilanes in condensation is organochlorosilanes > organomethoxysilanes > organoethoxysilanes. The moderate reactivity of the ethoxysilane moieties of Ura-TES resulted in an attachment to the silicon substrate that was preferred over the self-condensation reaction of Ura-TES in the solution phase.

Figure 3 Change in the thickness of a Ura-TES self-assembled layer on the substrate as a function of the immersion time. The layer thickness was determined by ellipsometry measurements. The substrate was immersed in a cyclohexanone solution of 2 mM Ura-TES.

Figure 4 Relationship between immersion time and the amount of uracil immobilized onto the substrate. The substrate was immersed in a cyclohexanone solution of 2 mM Ura-TES.

XPS is a quantitative technique for directly analyzing the surface composition of polymer particles and films. The XPS analysis provides important information about the surface composition and chemical states of films from the top of the film to a depth of ~10 nm. Using XPS, we investigated the chemical structure of the Ura-TES self-assembled layer on a substrate. The experimental and theoretical area ratios of the component peak of the Ura-TES self-assembled layer are summarized in Table 1. The experimental area ratios of the component peaks were determined by XPS analysis, and the theoretical area ratios were calculated from the chemical structure of the original Ura-TES and self-assembled Ura-TES in which the ethoxy groups were eliminated by an attachment reaction to the substrate. Three peaks were found from fitting the curve of the Ura-TES self-assembled layer's XPS C_{1s} spectra: Peak 1, which occurred at 285.0 eV, was assigned to $-\text{CH}_2-$ and $-\text{C}-\text{NH}-$, Peak 2, which occurred at 286.5 eV, was assigned to $-\text{C}-\text{O}-$ and Peak 3, which occurred at 288.1 eV, was assigned to $>\text{C}=\text{O}$. Compared with the theoretical area ratios of the original Ura-TES, the experimental area ratios of the component peaks corresponded well with the theoretical area ratios of the self-assembled Ura-TES without the ethoxy groups, indicating that most of the ethoxysilanes of Ura-TES underwent the hydrolysis and condensation reaction to form a self-assembled layer on the substrate surface.

The thickness of the Ura-TES self-assembled layers formed on the silicon substrate was measured using an ellipsometer. Figure 3 shows the effect of the immersion time on the thickness of the Ura-TES self-assembled layer when the silicon substrate was immersed in a cyclohexanone solution of Ura-TES. The thickness of the Ura-TES self-assembled layer increased significantly to ~9 nm as the immersion time increased to ~10 h and became constant for immersion times longer than 10 h. These results indicate that the substrate surface was completely covered with the Ura-TES self-assembled layer after being immersed for ~10 h. To determine the self-assembly of Ura-TES from the viewpoint of surface modifications, we measured the amount of uracil immobilized on the silicon substrate. The amount of uracil on the substrate was determined by measuring the amount of detached Ura-TES after the binding between Ura-TES and the substrate surface was hydrolyzed by HCl. The relationship between the immersion time and the amount of uracil immobilized on the substrate is shown in Figure 4. The amount of immobilized uracil increased monotonously as the immersion time increased when the silicon substrate was immersed in a cyclohexanone solution of Ura-TES. As shown in Figure 3, however, the thickness of the Ura-TES self-assembled layer remained constant at 9 nm when the substrate was immersed for >10 h, indicating that the uracil moieties of the Ura-TES self-assembled layer existed within the layer in addition to at the surface. Thus, using Ura-TES, uracil, which has multiple hydrogen bonding sites, can be easily organized on the substrate surface without a complicated process. Simple and versatile processes such as self-assembly are very useful for introducing functional groups to the surfaces of various materials.

Figure 5 Schematic of the self-assembly of Ura-TES onto a silicon substrate. A full color version of this figure is available at *Polymer Journal* online.

The self-assembly of Ura-TES on a substrate surface can be explained by the tentative schematic shown in Figure 5. The ethoxysilane moieties of Ura-TES are spontaneously subjected to hydrolysis before condensation in the presence of a small amount of water.^{2,7,20} Water for the hydrolysis, followed by the generation of reactive silanol groups, comes from several sources. For example, water may come from the atmosphere or be present on the substrate surface. The hydrolysis of Ura-TES in a solution containing a small amount of water is followed by a condensation reaction in the solution phase, resulting in the formation of Ura-TES aggregates. In a cyclohexanone solution without water, however, Ura-TES is hydrolyzed by a small amount of water that is adsorbed on the substrate surface, followed by condensation and layer formation on the substrate. Furthermore, the reactivity of ethoxysilane is typically lower than that of chlorosilane. The moderate reactivity of the ethoxysilane moieties of Ura-TES also causes the formation of the self-assembled layer on the substrate to be preferred over the formation of aggregates in the solution phase. For these reasons, the uracil-concentrated surface was easily prepared by the self-assembly of Ura-TES. Thus, using Ura-TES synthesized by a simple method, uracil that has multiple hydrogen bonding sites can be easily attached to a solid substrate.

Adsorption of adenine on the Ura-TES self-assembled surface

Nucleobases with multiple hydrogen bonding sites have the most important role in the complementary molecular recognition of DNA and RNA. Uracil, which is a nucleobase of RNA, can interact with the

Figure 6 Relationship between the amount of uracil immobilized on the substrate and the amount of adenine adsorbed onto a Ura-TES self-assembled layer. The substrate on which Ura-TES was self-assembled was immersed in an aqueous solution of 10 mM adenine.

adenine of a complementary nucleobase by the formation of two hydrogen bonds. We investigated the adsorption of adenine onto a uracil-concentrated surface because the surface is easily prepared by the self-assembly of Ura-TES. First, atomic ratios on the substrate surface were measured by XPS before and after the uracil-immobilized substrates were immersed in an aqueous solution of adenine for 24 h. The XPS measurements revealed that the experimental atomic ratios of oxygen to nitrogen (O/N) on the surface before and after the immersion were 6.24 and 5.79, respectively. However, the self-assembled uracil and free adenine's theoretical O/N atomic ratios, which can be determined based on their molecular structure, are 1.67 and 0, respectively. A large number of oxygen atoms exist on the surfaces of standard silicon wafers. Owing to the Ura-TES self-assembled layers were very thin, the experimental O/N atomic ratios determined by the XPS measurements were based on the oxygen atoms of the silicon substrate as well as the uracil self-assembled layers and adsorbed adenine, causing the experimental atomic ratios to be significantly higher than the theoretical atomic ratios. However, it should be noted that the experimental O/N atomic ratio decreased from 6.24 to 5.79 when the uracil-immobilized substrate was immersed in the aqueous solution of adenine. Owing to the theoretical O/N atomic ratio of free adenine is zero, a decrease in the atomic ratio by immersion suggests that adenine was adsorbed on the Ura-TES self-assembled layer. Furthermore, to quantitatively evaluate the adenine adsorption onto the Ura-TES self-assembled layer, we measured the amount of adsorbed adenine as a function of the immersion time. Figure 6 shows the relationship between the amount of uracil immobilized on the silicon substrate and the amount of adenine adsorbed onto the uracil-concentrated surface. As the amount of uracil immobilized on the silicon substrate increased, the amount of adsorbed adenine increased significantly up to $\sim 5 \text{ mol m}^{-2}$. It should be noted that the adenine adsorption on a uracil-concentrated surface became constant as the amount of immobilized uracil exceeded 7 mol m^{-2} . This result implies that adenine interacted with $\sim 70\%$ of the uracil immobilized on the silicon substrate.

As shown in Figure 7, a uracil molecule typically interacts with an adenine molecule by two hydrogen bonds. However, because Ura-TES was self-assembled on the silicon substrate, the uracil moieties were crowded on the surface. It is difficult for all of the crowded uracil moieties on a substrate surface to interact with adenine molecules. As shown in Figure 3, the thickness of the Ura-TES self-assembled layer remained constant at 9 nm when the silicon substrate was immersed for >10 h. However, Figure 4 demonstrates that the amount of uracil immobilized on the substrate increased to 7 mol m^{-2} after being immersed for 10 h; furthermore, the amount increased monotonously as the immersion time increased over 10 h, suggesting that the uracil moieties of the Ura-TES self-assembled layer existed within the layer as well as at the top surface. Adenine cannot approach uracil moieties within the layer; it is only adsorbed onto uracil moieties at the top surface. Furthermore, the adenine adsorption onto a uracil-concentrated

Figure 7 Schematic of the adsorption of adenine onto the uracil-concentrated surface.

substrate occurs in a heterogeneous system despite the fact that the molecular interaction between uracil and adenine in an aqueous solution occurs in a homogeneous system. Thus, adenine interacted with ~70% of the uracil immobilized on the substrate. As described previously, Ura-TES can be easily synthesized by a simple reaction of bromouracil with amino-terminated organosilane without solvents. Because many hydroxylated surfaces are easily surface modified by the self-assembly of Ura-TES, we can prepare various devices with unique surfaces that recognize target nucleobases. Our method using the self-assembly of nucleobase-terminated organosilanes is useful in fabricating nucleobase-concentrated surfaces for various biomedical applications, such as sensing, bioimaging and DDS.

CONCLUSIONS

We have demonstrated a simple method for synthesizing nucleobase-terminated organosilanes that only consists of a reaction of bromouracil with amino-terminated organosilane without solvents. When the silicon substrate was immersed in a cyclohexanone solution of the resultant uracil-terminated organosilane, a uracil-concentrated thin layer was formed on the silicon substrate by the self-assembly of organosilane. The AFM observations and ellipsometry revealed that the thickness of the self-assembled layer on the substrate increased up to ~9 nm as the immersion time increased. The self-assembly of a uracil-terminated organosilane on the substrate resulted from the hydrolysis of silane moieties in the presence of a small amount of water on the substrate surface, followed by the reaction of the resultant silanol groups with the hydroxyl groups of the substrate surface. Adenine complementary to uracil was effectively adsorbed onto the uracil-concentrated surface of the self-assembled layer in water. The adsorption of adenine onto the uracil-concentrated surface was based on the complementary hydrogen bonding between uracil and adenine. Thus, biomolecule-terminated organosilanes will contribute significantly to the development of useful bioconjugated devices for diagnostic sensors and imaging systems. Knowledge gained from the synthesis of nucleobase-terminated organosilanes provides a basis for the development of biomolecule self-assembled layers and permits further insight into structural design for the fabrication of bioconjugated devices. Although the novel nucleobase-terminated organosilane requires further research work into possible applications, it will likely become an important bioconjugated molecule for constructing sensing systems, bioimaging systems and DDS.

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