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Biotinylation of Silicon and Nickel Surfaces and Detection of Streptavidin as Biosensor

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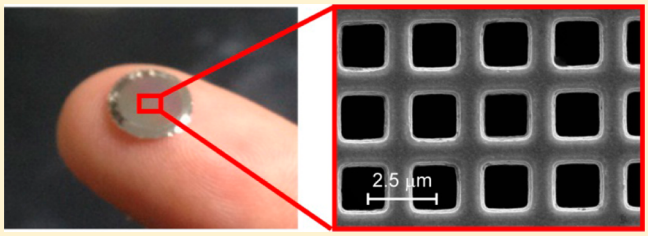
Supporting Information

ABSTRACT: The availability of metal mesh device sensors has been investigated using surface-modified nickel mesh. Biotin was immobilized on the sensor surfaces consisting of silicon and nickel via a thiol−ene click reaction, known as the Michael addition reaction. Biotinylation on the maleimidated surface was confirmed by X-ray photoelectron spectroscopy. The binding of streptavidin to the biotinylated surfaces was evaluated using a quartz crystal microbalance and a metal mesh device sensor, with both techniques providing similar binding constant value. The recognition ability of the biotin immobilized using the thiol-maleimide method for streptavidin was comparable to that of biotin immobilized via several other methods. The adsorption of a biotin conjugate onto the streptavidin-immobilized surface via the biotin−streptavidin−biotin sandwich method was evaluated using a fluorescent microarray, with the results demonstrating that the biological activity of the streptavidin remained.



INTRODUCTION

Biosensors are composed of sensing devices that contain molecular recognition interfaces for a particular biomolecule. Novel biosensing and screening systems can be created by surface modification of devices using biomolecules, in which the biochemical reaction is directly detected by an integrated electronic circuit. Surface immobilization techniques have been developed to detect a number of biomolecules, including antibodies, enzymes, lectins, saccharides, allergens, and DNA molecules.[1] In biosensors, it is imperative that the original biological activity is retained after biomolecule immobilization.

In the 1960s, metal mesh devices (MMDs) with two-dimensional periodic structures were found to exhibit frequency selectivity, and could be used as band-pass filters.[2] The electric field in these devices was localized around the surface of MMD. MMDs have been applied in the development of biosensing devices for a variety of different biomolecules, including DNA molecules and proteins, with characterization occurring in the terahertz (THz) region.[3−7] The MMD sensor has attracted considerable attention as a label-free detection technique, and is an alternative to quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) sensors. The MMD sensor allows for a low detection limit (>500 pg mm^{-2}), which is similar to QCM. The biggest advantage of MMD sensing is the ability to analyze a wide range of different sizes (from nanosized proteins to microsized polymer films). The localized region of the electric field is controlled by the selection of MMD periodic size and light source frequency.[8] As a result, the MMD sensing technique, which fits into the target size, can be established. Wide-ranging electromagnetic waves, such as microwave to visible light, are operated in the MMD sensing on an identical principle. Because the transmittance property of the MMD depends little on the metal species, the device can be manufactured from inexpensive materials. The MMD sensor is more rapid and facile than QCM and SPR. In addition, the MMD measurement is hardly affected by temperature change, compared with QCM measurement.

The MMD sensor measures changes in the transmittance spectrum, which result from changes in the complex refractive index of the nearby surface. Because the thickness of electric field is inversely proportional to the frequency, the use of the MMD in the mid-infrared frequency range improves the sensitivity of the biosensing device. To successfully operate the MMD in the mid-infrared frequency range, it is necessary for the MMD to have a small periodic structure. An MMD sensor with a periodic structure, constructed from square apertures with 6.5 μm of a grid interval and 4.5 μm of an opening length, was recently developed to operate at 40 THz.[7] The intensity of the electric field decreases exponentially with the distance from the surface of the MMD. In previous MMD sensing studies, the

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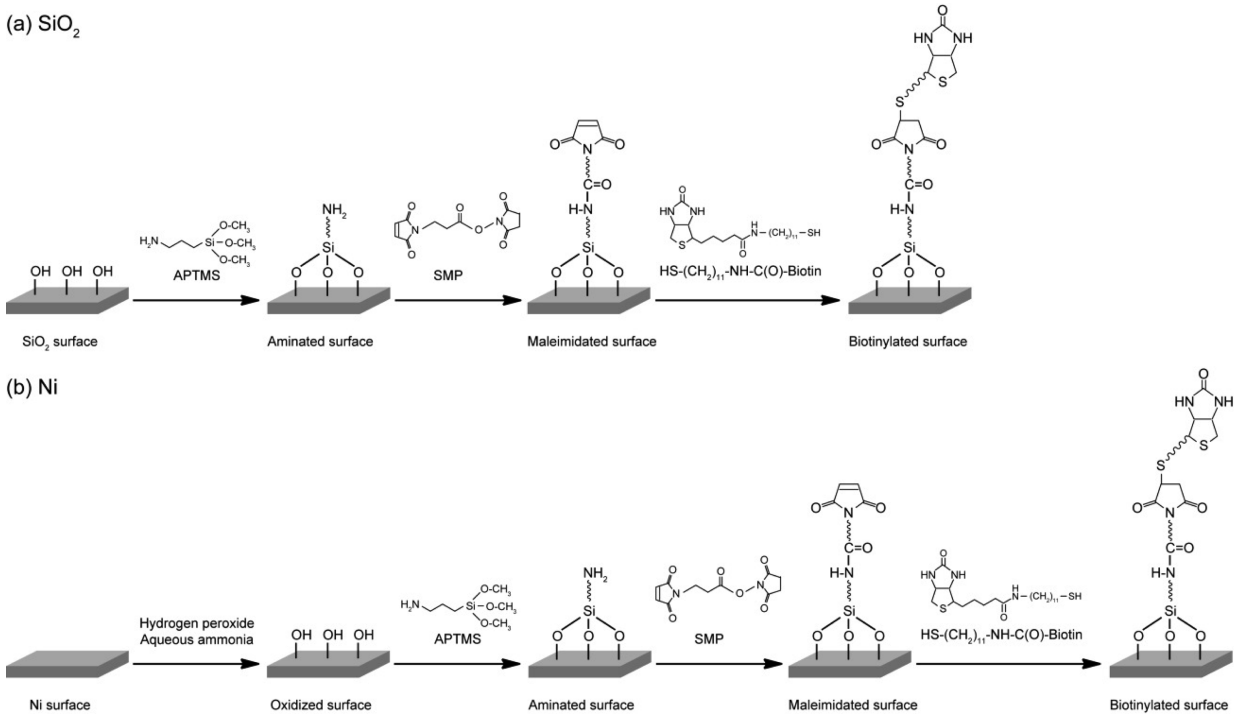

Figure 1. Preparation of biotinylated surface via amination and maleimidation using (a) SiO_2 and (b) Ni.

analytes were physically attached to the MMD surface.[4,6,7] In an alternative method, the analytes were trapped in a membrane filter placed near the MMD surface.[3,5] In these cases, the distance between the MMD surface and the analyte was relatively large. To achieve effective biosensing levels, the analytes should be adsorbed in the vicinity of the MMD surface. With this in mind, research toward the surface modification of MMDs using biomolecules as a recognition site is required.

Green et al.[9,10] characterized the noncovalent interactions of biotin−avidin and biotin−streptavidin complexes in solution, and reported high binding constants in the range of 10^{13}−10^{15} M^{-1} (M = mol L^{-1}). The formation of a complex between biotin and streptavidin has been widely applied in a variety of different research areas, including biosensors,[11] bioseparations,[12] bioreactors,[13] and drug delivery systems,[14] where the biotin−streptavidin interaction acted as a linker between the biomolecule and the substrate. When streptavidin is directly immobilized onto a substrate in a preliminary step toward eventually linking a biotin conjugate to a substrate, its activity can be attenuated because of denature. The immobilization of streptavidin via biotinylation, according to the biotin−streptavidin−biotin sandwich method, would therefore represent a more effective method than that of direct immobilization. The streptavidin-immobilized surface could also expand the application of the MMD sensor to the detection of enzymatic reactions, antigen−antibody interactions, and DNA hybridization processes.

In the current study, the surfaces of sensor chips consisting of silicon (Si) or nickel (Ni) were biotinylated to bind with streptavidin, using a thiolated biotin reagent. The thiolated biotin was immobilized via the surface maleimidation, as shown in Figure 1. The maleimidated surface was prepared via an amide condensation reaction with an amine-containing silane coupling reagent.[15] The binding of streptavidin to the biotinylated surface was evaluated using the QCM and MMD sensors. High frequency (100 THz)-operating Ni mesh was used to fit into the nanosized streptavidin. Furthermore, the association ability of streptavidin on biotinylated surface against the biotin conjugate was determined from fluorescent microarray measurements.

EXPERIMENTAL SECTION

Reagents and Substrates. 3-Aminopropyl trimethoxysilane (APTMS, Azmax Co., Chiba, Japan) and *N*-succinimidyl-3-maleinimidopropionate (SMP, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) were used for the preparation of the maleimidated surface. Thiol-terminated biotin (HS-$(CH_2)_{11}$-NH-C(O)-biotin, ProChimia Co., Gdansk, Poland) was used for the biotinylation of the surface. Streptavidin (Wako Pure Chemical Industries Ltd., Osaka, Japan) was used as a protein probe.

Si wafers, glass slides (printed with highly water-repellent mark, TF2404, Matsunami Glass Ind. Ltd., Osaka, Japan), SiO_2-sputtered oscillators for the QCM sensor (effective surface area: 4.9 mm^2, Initium Inc., Tokyo, Japan), and Ni meshes for the MMD sensor (effective surface area: 67 mm^2) were used in the experiments. The Ni mesh for the MMD had 1.8 μm opening length, 1 μm thickness, and 2.6 μm grid interval (Figure S1). The working frequency of the Ni mesh was approximately 100 THz. The Au oscillator of the QCM (effective surface area: 4.9 mm^2, Initium Inc., Tokyo, Japan) and the SIA kit Au (Biacore AB, Uppsala, Sweden) were used to form the self-assembled monolayer. The surfaces of the Si wafers, glass slides, and Ni meshes were sequentially washed in acetone, ethanol, and water. Following a 30 min period of UV/O_3 treatment, the surfaces were activated in a mixture of hydrogen peroxide and aqueous ammonia at 60 °C for 20 min. The surface of the SiO_2-sputtered oscillator in the QCM cell was cleaned with a drop of piranha solution (sulfuric acid:hydrogen peroxide = 3:1), instead of UV/O_3 treatment.

Biotinylation of Si and Ni Surfaces. The Si wafer, glass slide, Ni mesh, and SiO_2-sputtered oscillator were maleimidated according to a method described previously in the literature.[15] With the exception of the SiO_2-sputtered oscillator, the maleimidated substrates were quickly immersed in an ethanol solution of HS-$(CH_2)_{11}$-NH-C(O)-biotin (1.2 mmol L^{-1}), and incubated in the dark at room temperature for 12 h. The resulting biotinylated surfaces were then washed with ethanol and dried. The amination, maleimidation, and biotinylation processes were

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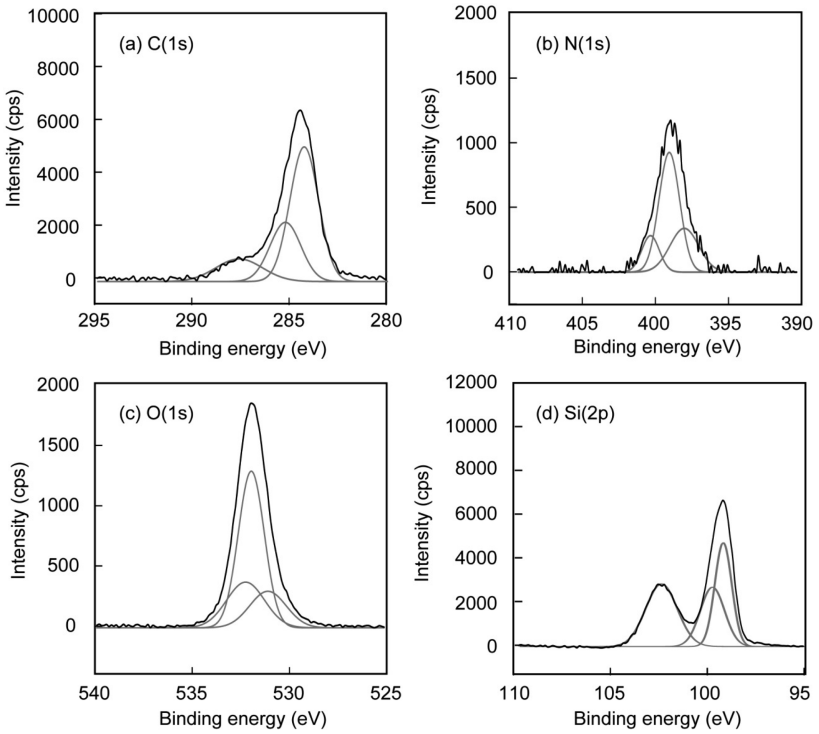

Figure 2. X-ray photoelectron spectra of the biotinylated Si wafer; (a) C(1s), (b) N(1s), (c) O(1s), and (d) Si(2p).

confirmed by C(1s), N(1s), S(2p), Si(2p), and Ni(2p) X-ray photoelectron spectroscopy (XPS, Quantum2000, Physical Electronics, Inc., Chanhassen, MN, USA). The XPS spectra were calibrated using the peak corresponding to C−C at 284.6 eV. The resulting XPS spectra were peak-divided using a commercially available software Peak Fit (v4.12, Systat Software Inc., San Jose, CA, USA).

Measurement of QCM Using the Biotinylated SiO_2 Oscillators. The amount of biotinylation on the oscillator was determined using QCM measurement (AFFINIXQ4, Initium Inc., Tokyo, Japan). The QCM cell containing the maleimidated oscillator was suffused with ethanol solution of HS-$(CH_2)_{11}$-NH-C(O)-biotin (1.2 mmol L^{-1}, 500 μL), and then incubated in the absence of light at room temperature for 12 h. The resulting biotinylated surface was then washed with ethanol and dried in a dryer at 40 °C. The surface density (Δm, ng cm^{-2}) of the biotin was then estimated from the frequency change at dry conditions phase (ΔF_{air}, Hz) before and after biotinylation process. The relationship between ΔF_{air} and Δm was defined by the Sauerbrey equation:[16]

$$\Delta F_{air} = -\frac{2NF_0^2}{\sqrt{\rho\mu}}\Delta m \quad (1)$$

$$\Delta m \cong -0.62 \times \Delta F_{air} \quad (2)$$

where N, F_0, ρ, and μ are the harmonic overtone, fundamental resonance frequency, crystal density, and elastic modulus of the crystal, respectively. The F_0 value of the apparatus was 27 MHz.

The adsorption behaviors of streptavidin on the biotinylated surface were quantitatively evaluated using the QCM measurements. The QCM cell containing the biotinylated oscillator was suffused with phosphate buffer solution, and left until the frequency reached equilibrium. The biotin−streptavidin reaction was initiated by the injection of the streptavidin solution into the QCM cell. Following an incubation period of 3 h, the oscillator was washed sequentially with the buffer solution and water, and then dried in a dryer at 40 °C. The frequency of the QCM was determined at dry conditions. The Δm value of the streptavidin was estimated from the ΔF_{air} values before and after the adsorption of streptavidin using eq 2. The maximum capacity of streptavidin on the biotinylated surface (Δm_{max}) and the apparent binding constant (K_a) between the biotin and the streptavidin on the surface were estimated from the Langmuir equation as follows:

$$\Delta F = \frac{C\Delta F_{max}}{1/K_a + C} \quad (3)$$

where C is the concentration of streptavidin in the QCM cell.

Measurement of MMD Sensor Using Biotinylated Ni Mesh. The adsorption behaviors of streptavidin on the biotinylated surface for the MMD sensor operated at 100 THz were evaluated in a similar manner to that described above for the QCM measurements. The biotinylated Ni mesh was immersed in streptavidin solutions at a variety of different concentrations. Following a 3 h incubation period in the absence of light, the meshes were washed with a sufficient quantity of water and dried. The transmittance infrared (IR) spectra of the Ni meshes at dry conditions were obtained before and after the adsorption of streptavidin using an IR spectrometer. A wavenumber resolution of 2 cm^{-1} was used together with an accumulation number of 8 measurements. The measurement time for one sample was less than 2 min. The frequency shift (Δf, THz) was estimated from the difference in the frequencies of the dipped peaks before and after the adsorption of streptavidin. The maximum frequency shift (Δf_{max}) and K_a values between the biotin and streptavidin on the surface were estimated from the Langmuir equation, by replacing ΔF for Δf, assuming that the Δf is proportional to the amount of adsorbed protein.

The streptavidin adsorbed was eluted from the biotinylated Ni mesh. The Ni meshes adsorbed with streptavidin at 10^{-1} g L^{-1} were immersed in the dithiothreitol-containing eluent, and then heated at 98 °C for 5 min. The eluted streptavidin was fractionated by electrophoresis with a poly(acrylamide) gel. The concentration of the eluted streptavidin was estimated from the total gray value on the electrophoretic band. The details of the processes for the elution and electrophoresis of streptavidin are described in the Supporting Information.

Formation of Biotin SAM on Au Surface and Determination of Streptavidin Adsorption Using QCM and SPR Sensors. The biotin SAM was formed on the Au surfaces. The Au surfaces were cleaned with piranha solution prior to their use. The QCM cell containing Au oscillator was suffused with ethanol solution of HS-

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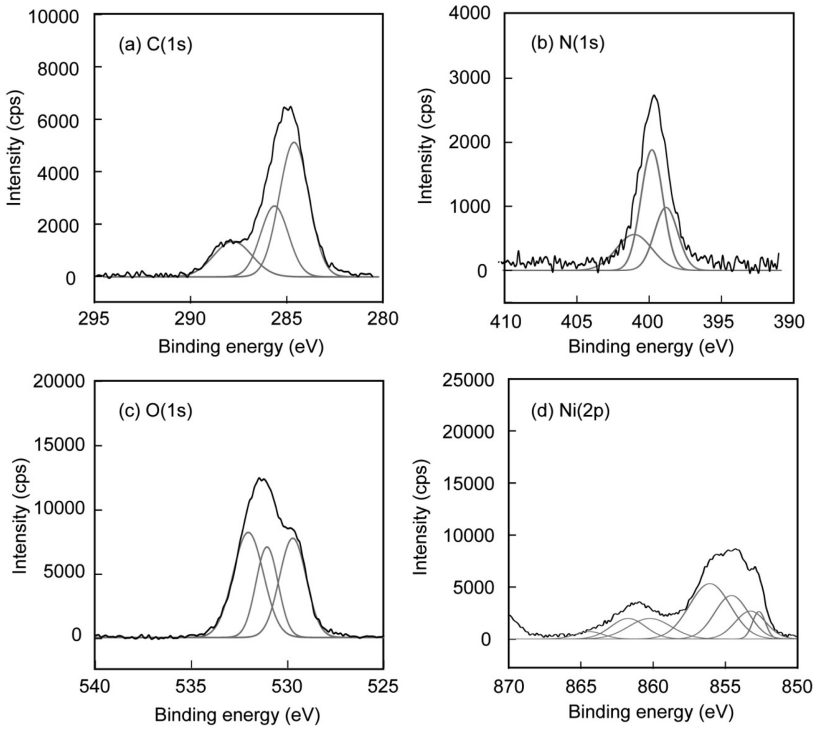

Figure 3. X-ray photoelectron spectra of the biotinylated Ni mesh; (a) C(1s), (b) N(1s), (c) O(1s), and (d) Si(2p).

Table 1. Atomic Percentages on Unmodified, Aminated, Maleimidated, and Biotinylated Si Wafers, Estimated from X-ray Photoelectron Spectra

	carbon		nitrogen		oxygen		silicon
	atom%	C/Si	atom%	N/Si	atom%	O/Si	atom%
control	26.2	0.68	0.4	0.01	34.9	0.91	38.5
aminated	30.7	0.86	1.8	0.05	31.9	0.90	35.6
maleimidated	35.8	1.14	3.0	0.10	29.8	0.95	31.4
biotinylated	35.6	1.26	3.5	0.12	32.7	1.16	28.2

$(CH_2)_{11}$-NH-C(O)-biotin (1.2 mmol L^{-1}, 500 μL), and then incubated in the absence of light at room temperature for 12 h. The resulting biotin SAM surfaces were washed with ethanol and dried. The Δm value of the biotin was estimated from the ΔF_{air} values before and after the formation of the biotin SAM. The Au chip of SPR was immersed in ethanol solution of HS-$(CH_2)_{11}$-NH-C(O)-biotin (1.2 mmol L^{-1}), and then incubated at room temperature for 12 h.

The interactions of the streptavidin and biotin SAM on the Au surfaces were detected by QCM and SPR. The procedure used to determine the streptavidin adsorption using QCM was the same that described above. For the SPR measurements, a Biacore X100 system (GE Healthcare, Little Chalfont, UK) running with a HBS-EP$^+$ buffer (10 mmol L^{-1} HEPES, pH 7.4, 3 mmol L^{-1} EDTA, 150 mmol L^{-1} NaCl, 0.05% Tween 20) was used. The baseline noise level of the system was <0.1 RU. A variety of different concentrations of streptavidin were injected at a continuous flow rate of 30 μL min^{-1} for 3 min. After each protein injection, the sensor chip surfaces were washed with an injection of 50 mmol L^{-1} NaOH for 1 min to dissociate any streptavidin interacting with the surface-bound biotin. All of the experiments were performed at 25 °C. The relationship between the SPR responses (resonance unit, abbreviated as RU) and the Δm values in the Biacore measurement can be expressed as follows:[17]

$$1.0\,\mathrm{RU} = 0.1\,\mathrm{ng\,cm^{-2}} \quad (4)$$

The kinetic constants of the interactions and the maximum responses of streptavidin were calculated using the BIAevalution software, which was adapted to the modified sensorgrams.

RESULTS AND DISCUSSION

Confirmation of Biotinylation on Si and Ni Surfaces. The occurrence of the amination and maleimidation processes on the Si and Ni surfaces were confirmed by XPS measurements (Figures S2 and S3). The XPS spectra of the C(1s), N(1s), O(1s), and Si or Ni(2p) on the biotinylated Si and Ni surfaces are shown in Figures 2 and 3, respectively. The peaks relating to the C−C bonds (284.6 eV), as well as the N−C and $-NH_2$ bonds (398.8 eV) were observed in Figures 2(a), 2(b), 3(a), and 3(b). The peak at 400.9 eV was assigned to the hydrogen-bonded and positively charged amines.[18,19] In both of the biotinylated surfaces, the intensities of the peaks corresponding to the C—N, C═O, and N—C═O bonds of the amide group at 285.3, 287.7, and 399.8 eV, respectively, were enhanced. In Figures 2(c) and 3(c), the peaks corresponding to O═C bond were observed at 531.0 eV. The peaks at 531.9 eV in Figure 2(c) and at 529.7 eV in Figure 3(c) were attributed to the O−Si and metal oxide bonds, respectively. The intensity of peak corresponding to metal oxide decreased following the biotinylation of the Ni surface. Two peaks were observed in Figure 2(d), where the higher peak corresponded to the Si−O bond. The second peak was divided into Si($2p^{1/2}$) at 99.8 eV and Si($2p^{3/2}$) at 99.3 eV. The peaks in Figure 3(d) were divided by the reference to the assignment reported by Grosvenor et al.[20] The intensities of the

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Table 2. Atomic Percentages on Unmodified, Aminated, Maleimidated, and Biotinylated Ni Meshes, Estimated from X-ray Photoelectron Spectra

	carbon		nitrogen		oxygen		nickel
	atom%	C/Ni	atom%	N/Ni	atom%	O/Ni	atom%
control	22.3	0.65	0.5	0.01	42.9	1.25	34.4
aminated	30.1	1.09	3.5	0.13	38.7	1.40	27.7
maleimidated	30.0	1.13	4.3	0.16	39.3	1.48	26.5
biotinylated	42.3	3.37	8.1	0.64	36.6	2.91	12.6

biotinylated surfaces in Si(2p) and Ni(2p) spectra became increasingly weak, indicating that the X-ray irradiation of the substrate surface was interfered by the immobilized biotin. The ratios of carbon, nitrogen, and oxygen atoms to the substrate atoms were enhanced as the surface modification process proceeded (Tables 1 and 2). The atomic percentages of nitrogen on the biotinylated surfaces were approximately two times greater than that on the aminated surfaces. These results confirmed that the biotin layer was successfully formed on the Si and Ni surfaces. The adsorption of streptavidin onto the biotinylated Si and Ni surfaces was also confirmed by XPS measurements (Figures S2 and S3).

Detection of Streptavidin Adsorbed on Biotinylated SiO_2 Oscillator Using QCM Sensor. To investigate the immobilization of biotin and the adsorption of streptavidin on the substrate surfaces, the QCM sensorgram was obtained. From the ΔF_{air} values of the QCM measurements, the Δm values of the amine and maleimide groups were estimated as 11.4 and 2.8 nmol cm^{-2}, respectively. According to the same process, the Δm value of the biotin was 0.32 nmol cm^{-2}, representing an adsorption of 1.93×10^{14} molecules cm^{-2}. The number of thiolated biotin molecules per unit area has been reported to be 4.23×10^{14} molecules cm^{-2}.[21] Therefore, in this particular case, 47% of the surface was biotinylated. These Δm values were of a similar order to the molar ratio values estimated from the XPS measurement (see the Supporting Information).

Following the stabilization of the ΔF value of the QCM with the biotinylated oscillator at dry conditions, the QCM cells were filled with phosphate buffer solution (Figure S5). Streptavidin solutions were injected into the frequency-stabilized QCM cells. The ΔF values was negatively changed, and then reached equilibrium within 30 min, indicating that the streptavidin was rapidly bound to the biotin on the surface. Following the adsorption of streptavidin on the biotinylated oscillators, the QCM measurements were performed at dry conditions. The ΔF_{air} values of streptavidin adsorbed onto the unmodified and biotinylated oscillators are shown in Figure 4a. The unmodified oscillator did not provide any adsorption of streptavidin, whereas significant levels of adsorption were observed with the biotinylated oscillator. The ΔF_{air} value of streptavidin on the biotinylated oscillator increased with increasing streptavidin concentration, and eventually reached 680 Hz. This value was then converted into 420 ng cm^{-1} or 4.04×10^{12} molecules cm^{-2}. The monovalent surface density of streptavidin has been reported to be approximately 5×10^{12} molecules cm^{-2} at close packing, using a streptavidin size of $4.5 \times 4.5 \times 5.5$ nm^3.[22] The surface coverage of streptavidin on the SiO_2 oscillator of the QCM was therefore approximately 80%, suggesting that a monolayer of streptavidin was formed on the surface. The molar ratio between the immobilized biotin and streptavidin was 48:1, which was close to the value estimated from XPS results (see the Supporting Information).

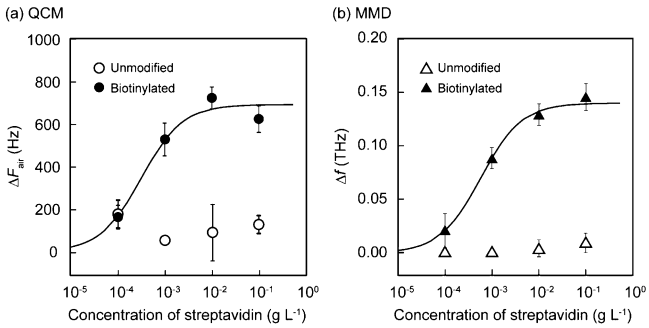

Figure 4. Detections of streptavidin using (a) the biotinylated SiO_2 oscillator of the quartz crystal microbalance and (b) the biotinylated Ni mesh of metal mesh device. The error bars are the standard deviation determined for at least two times. The solid lines represent the curve fitted on the basis of eq 3. $R^2 = 0.9821$ (QCM) and $R^2 = 0.9950$ (MMD).

Although all of Langmuir's theories should not be fulfilled in the biotin–streptavidin interaction, several researchers have applied Langmuir model to calculate K_a.[23−27] Langmuir model was also applied in the current study for fitting the data between the ΔF_{air} value and the streptavidin concentration. In fact, in the current study, the binding behavior of streptavidin on the biotinylated SiO_2 oscillator surface resembled the Langmuir model quite closely. The K_a value between the biotin and streptavidin on the SiO_2 oscillator surface in the current study was 1.7×10^8 M^{-1}. This value was lower than the K_a value between the biotin and streptavidin in solution, which was 10^{13} M^{-1}. Not all of the recognition sites of the tetrameric streptavidin were used to bind with the immobilized biotin because of steric hindrance.[25] Based on these results, it was estimated that the streptavidin was monovalently interacting with the biotin immobilized on the surface. The K_a value for the interaction of the streptavidin with the biotin immobilized via maleimide–thiol reaction closely resembled that of the monomeric avidin subunit with biotin (i.e., 2×10^7 M^{-1}).[28]

Detection of Streptavidin Adsorbed on Biotinylated Ni Mesh Using MMD Sensor. The MMD sensor was measured to estimate the interaction between the biotin and the streptavidin on the Ni surface. The original Ni mesh possessed a lattice-shaped morphology with 1.8 μm open length. The transmittance spectrum of each Ni mesh was rapidly obtained within 2 min. The dip was observed at 90–100 THz in the transmittance spectrum of the original Ni mesh (Figure S1). The reduction in the refractive index on the metal mesh provided a red shift of frequency in the far- and mid-infrared ranges, with the magnitude of the shift being proportional to the amount of material attached to the mesh. The shift magnitude of the dip in the transmittance spectra of the Ni mesh adsorbed with streptavidin was dependent on the streptavidin concentration (Figure S6). The Δf values of the streptavidin adsorbed onto the unmodified and biotinylated Ni

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meshes are shown in Figure 4b. The unmodified Ni mesh was not adsorbed with streptavidin, whereas the biotinylated Ni mesh was strongly adsorbed. The Δf value of the streptavidin on the biotinylated Ni mesh increased with increasing the streptavidin concentration, and eventually reached 0.14 THz. The amount of streptavidin adsorbed was determined by electrophoresis at a concentration of 10^{-1} g L^{-1} on the biotinylated Ni surface (Figure S7), and estimated to be 170 ng cm^{-2}. The K_a value between the biotin and the streptavidin on the surface of the Ni mesh was estimated from the Δf value and the streptavidin concentration using Langmuir equation. The value was 9.1×10^7 M^{-1}, which was consistent with that on the surface of the SiO_2-sputtered oscillator estimated from the QCM measurement. These results indicated the relevance of the MMD-based biosensor.

Detection of Streptavidin Adsorbed on Biotin SAM-Formed Au Surfaces Using QCM and SPR Sensors. The adsorption ability of the biotin immobilized via the thiol–Au interaction was determined by the QCM and SPR sensors, to compare that of the biotin immobilized via the thiol-maleimide reaction. The Δm value of the biotin SAM estimated from the ΔF_{air} value of the QCM measurements was 0.63 nmol cm^{-2}, representing approximately 90% surface coverage. The ΔF_{air} value of the QCM measurement as well as the SPR sensorgrams observed during the adsorption of streptavidin onto the biotin SAM Au surfaces are shown in Figure 5, respectively. The K_a values of the streptavidin with the biotin SAM on the Au oscillator of the QCM and the Au chip of the SPR were 8.8×10^6 and 2.2×10^7 M^{-1}, respectively.

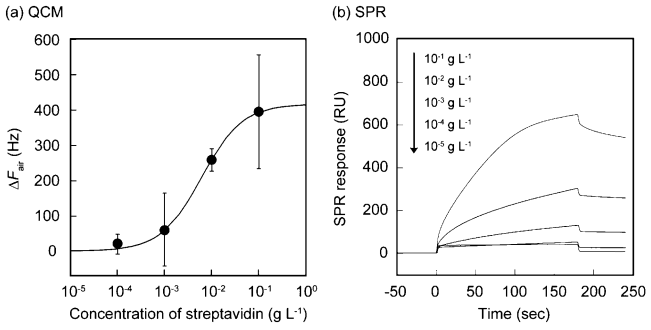

Figure 5. Detections of streptavidin using (a) the biotin self-assembled monolayer Au oscillator of the quartz crystal microbalance and (b) the biotin self-assembled monolayer Au chip of the surface plasmon resonance. The error bars are the standard deviation determined for two times. The solid line in (a) represents the curve fitted on the basis of eq 3. $R^2 = 0.9978$ (QCM).

The Δm_{max} value of streptavidin and the K_a value between the biotin and the streptavidin on the Si, Ni, and Au surfaces are summarized in Table 3. From the Δm_{max} values of streptavidin, the surface coverage values of streptavidin on the SiO_2 oscillator (QCM), Ni mesh (MMD), Au oscillator (QCM), and Au chip (SPR) were estimated to be aproximately 80%, 30%, 50%, and 10%, respectively, suggesting that streptavidin was adsorbed onto these surfaces as a monolayer. The K_a values were within the range of values previously reported in other publications, including biotin/ethylene glycol SAM,[27,29] biotin immobilized via amide condensation,[23,25,26] biotinylated dendrimer,[24] and biotin lipid,[30] with K_a values in the range of 10^6–10^8 M^{-1}. Therefore, the biological activity of biotin immobilized via the thiol-maleimide reaction was

Table 3. Surface Density of Biotin, Maximum Capacity of Streptavidin, and Apparent Binding Constant between Biotin and Streptavidin on the Si, Ni, and Au Surfaces

surface		preparation technique	Δm_{max} of streptavidin[a] (ng cm^{-2})	K_a (M^{-1})
Si	QCM	Thiol-maleimide	420[b]	1.7×10^8
Ni	MMD	Thiol-maleimide	170	9.1×10^7
Au	QCM	Thiol-Au	260[b]	8.8×10^6
Au	SPR	Thiol-Au	64[c]	2.2×10^7

[a]Theoretical surface density of streptavidin is ca. 500 ng cm^{-2}, when streptavidin is monovalently bound onto surface. [b]Δm of streptavidin in the measurement of QCM was estimated from eq 2. [c]Δm of streptavidin in the measurement of SPR was estimated from eq 4.

comparable to the activities achieved using other techniques. The recognition capacities and abilities of the biotin SAM Au surfaces against streptavidin were less than those of the biotinylated Si and Ni surfaces. This difference was attributed to the high surface density of biotin, as reported by Jung et al.,[31] who found that the immobilized biotin had suitable surface density for binding to streptavidin at 10–40%, and that recognition against streptavidin decreased with increasing surface density of the immobilized biotin above 40%. The surface of the Ni mesh device can be modified according to a variety of different methods, and these surface modifications lead to preparation of a specific biosensor. Moreover, when the use of the biotin–streptavidin–biotin sandwich method (Figure S8) for the immobilization of the biotin conjugate was investigated by fluorescent microarray, the biotin conjugate with low concentration (more than 1 nmol L^{-1}) was adsorbed on the streptavidin-immobilized surface (Figure S9). These results suggested that the high recognition ability of the immobilized streptavidin toward biotin remained intact. Biotin-labeled biomolecules can be successfully immobilized on the Ni mesh using the biotin–streptavidin–biotin sandwich method. Research in our laboratory is now focused exclusively on the practical application of MMD biosensing using Ni meshes modified with biomolecules, such as antibodies and DNA molecules, via the sandwich method.

CONCLUSIONS

In summary, the MMD sensor can be used as an alternative and a novel technique to QCM and SPR for the detection of biomolecular analytes with high levels of sensitivity. Streptavidin was directly adsorbed onto the sensor chip of the MMD without membrane support through the biotinylation of the Ni mesh. The binding constant between the streptavidin and the immobilized biotin, as determined using MMD sensing, was consistent with the value determined using the QCM measurement. Furthermore, the streptavidin adsorbed onto the biotinylated surface exhibited an ability to bind to the biotin conjugate, expecting that biotin-labeled proteins and DNA molecules can be immobilized on the surface of the Ni mesh. The MMD biosensor could therefore be applied in the field of medical testing.

ASSOCIATED CONTENT

Supporting Information

Details of the physical properties and transmittance spectra of the 100 THz-operating Ni mesh, the confirmation of amination and maleimidation on Si and Ni surfaces, the frequency changes during streptavidin adsorption on the biotinylated SiO_2-

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oscillator of the QCM cell, the amount of streptavidin adsorbed on the biotinylated Ni mesh determined by electrophoresis, transmittance IR spectra of the Ni mesh before and after the adsorption of streptavidin, and the adsorption of the biotin conjugate on the streptavidin-immobilized glass slide. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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