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Thermoresponsive protein adsorption of poly(*N*-isopropylacrylamide)-modified streptavidin on polydimethylsiloxane microchannel surfaces

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ABSTRACT

The control of protein adsorption on microchannel surfaces is important for biosensors. In this study, we demonstrated protein adsorption method that is controlled through temperature change, *i.e.*, thermoresponsive protein adsorption, on polydimethylsiloxane (PDMS) microchannel surfaces using a thermoresponsive polymer, poly(*N*-isopropylacrylamide) (PNIPAAm). To provide general protein adsorption control method, we adopted biotin–streptavidin chemistry and synthesized streptavidin covalently modified with PNIPAAm (PNIPAAm–StAv). Modification of streptavidin, a hydrophilic protein, with PNIPAAm induced successful thermoresponsive adsorption on a PDMS microchannel surfaces: PNIPAAm–StAv adsorbed at 37 °C and desorbed at 10 °C on the surfaces. We also demonstrated the thermoresponsive adsorption of biotinylated immunoglobulin G (IgG-b) using PNIPAAm–StAv. Conjugation of IgG-b with PNIPAAm–StAv induced successful thermoresponsive IgG-b adsorption on PDMS. Modification of PDMS surfaces with PNIPAAm reduced physical adsorption of the partially hydrophobic IgG-b on the surface and contributed to the high-contrast thermoresponsive adsorption of IgG-b: less than 1% of the IgG-b adsorbed at 37 °C was detected after the PNIPAAm–PDMS surface was washed at 10 °C. The controllable adsorption of this system is expected to be applied to the regeneration of biosensor chips and to on-chip protein manipulation.

1. Introduction

The control of protein adsorption on microchannel surfaces is important for improving sensitivity, accuracy, and reproducibility of biosensors and biochips (Kannan et al., 2006; Larson et al., 2005; Lee et al., 2003; Shim et al., 2007). Microchannels on which antibodies are immobilized have been used for immunoassays (Malmstadt et al., 2004), cell separation (Chang et al., 2005; Plouffe et al., 2008) and cell analysis (Nagrath et al., 2007). For these applications, controlled and active protein adsorption, in which the adsorbed proteins' surface chemistry changes in response to on-chip stimuli such as applied voltages, heat and light, provides greater functionality of the devices (Huber et al., 2003): regeneration of biosensors, on-chip protein manipulation and on-demand manipulation of cells.

Poly(*N*-isopropylacrylamide) (PNIPAAm) is well-known thermoresponsive polymer and has been applied to the thermoresponsive physicochemical property change of proteins and surfaces. PNIPAAm change its structure in response to changing temperatures in aqueous solutions (Heskins and Guillet, 1968). Polymer chains of PNIPAAm hydrate to form expanded structures in water at temperatures below the polymer's lower critical solution temperature (LCST) of 32 °C. At temperatures above the LCST, the PNIPAAm chains are dehydrated, forming compact structures that can aggregate and precipitate from solution. Proteins modified with PNIPAAm are also known to precipitate in aqueous solution at temperatures above the LCST (Chen and Hoffman, 1990; Malmstadt et al., 2003; Takei et al., 1993b, 1994). Additionally, the wettability of surfaces modified with PNIPAAm changes in response to temperature changes (Cheng et al., 2005; Ebara et al., 2006; Yakushiji et al., 1998): the surfaces become hydrophobic at the temperature above the LCST.

Streptavidin (StAv) is a 53-kDa tetrameric protein produced in nature by the bacterium *Streptomyces avidinii* (Green, 1990). Each of the four StAv monomers binds one molecule of the vitamin biotin with extraordinarily high affinity: the StAv–biotin interaction is one of the strongest noncovalent interactions in biochemistry, with an association constant (K_a) of 10^{14} to 10^{15} M^{-1} (Green, 1990). This strong interaction has been exploited as general method to immobilize functional molecules on biosensors: various biosensor component molecules and surfaces can be easily biotinylated,

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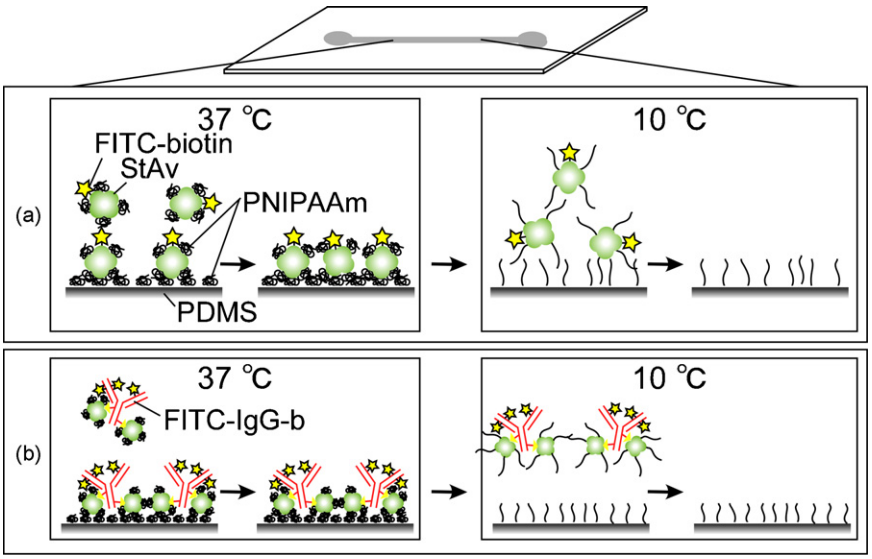

Fig. 1. Schematics of thermoresponsive protein adsorption on a modified PDMS surface. (a) Thermoresponsive adsorption of PNIPAAm–StAv on a PNIPAAm–PDMS surface. (b) Thermoresponsive adsorption of biotinylated FITC-IgG (FITC-IgG-b) conjugated with PNIPAAm–StAv on a PNIPAAm–PDMS surface.

permitting interaction between StAv and the newly added biotin functionalities. In the previous study, StAv modified with PNIPAAm has also been reported to precipitate biotinylated biomolecules (Malmstadt et al., 2003).

Among the materials that can be used for biochips, polydimethylsiloxane (PDMS) is a flexible, durable, transparent and inexpensive polymer. PDMS is one of the most-utilized materials for fabricating biochips, and microstructures can be easily molded from photoresist templates by soft lithography (Duffy et al., 1998; Sia and Whitesides, 2003; Xia and Whitesides, 1998). However, because of hydrophobicity and susceptibility to nonspecific protein adsorption, the surface of PDMS often requires further modification for successful application of the material in biosensors and biochips. In the previous study, PDMS modified with PNIPAAm has also been reported to capture nanoparticles (Ebara et al., 2006).

In this report, we present a protein adsorption method controlled through temperature manipulation, *i.e.*, thermoresponsive protein adsorption, by modifying both proteins and surfaces with a thermoresponsive polymer (Fig. 1). To provide a general method applicable to various proteins, we synthesized StAv covalently modified with PNIPAAm (PNIPAAm–StAv). We also prepared PNIPAAm-modified PDMS (PNIPAAm–PDMS) microchannels by UV-induced graft polymerization. We demonstrated the thermoresponsive adsorption both of PNIPAAm–StAv and of biotinylated immunoglobulin G (IgG) conjugated with PNIPAAm–StAv on the PNIPAAm–PDMS microchannel surfaces (Fig. 1a and b, respectively). The effects of PNIPAAm polymer chains of PNIPAAm–StAv and of PNIPAAm–PDMS on the thermoresponsive protein adsorption were systematically examined by means of fluorescence microscopy for high-contrast thermoresponsive protein adsorption.

2. Materials and methods

2.1. Chemicals

N-Isopropylacrylamide (NIPAAm) was obtained from Tokyo Chemical Industry (Tokyo, Japan), purified by recrystallization from a mixed solution of toluene and hexane (4:1, v/v) and dried under vacuum. *N*-Hydroxysuccinimide (NHS) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) were purchased from Junsei Chemical (Tokyo, Japan). 2,2′-Azoisobutyronitrile (AIBN) was purchased from Tokyo Chemical Industry. 3-Mercaptopropionic acid (MPA) was purchased from Dojindo (Kumamoto, Japan). StAv was purchased from Wako Pure Chemical Industries (Osaka, Japan). Fluorescamine, benzophenone, sodium periodate, benzyl alcohol and fluorescein-isothiocyanate-labeled IgG (FITC-IgG) from human were all purchased from Sigma–Aldrich (St. Louis, MO, USA). FITC-labeled biotin (FITC–biotin) was obtained from Pierce (Rockford, IL, USA). Milli-Q water (Millipore, Billerica, MA, USA) was used for preparation of aqueous solutions. All other solvents were obtained from Wako and were used as received.

2.2. Surface modification of a PDMS plate and a PDMS microchannel with PNIPAAm by UV-induced graft polymerization

A flat PDMS plate for surface characterization was obtained by curing a 10:1 (w/w) mixture of PDMS prepolymer and curing agent (Sylgard 184, Dow Corning, Midland, MI) at 120 °C for 2 h. The plate was modified with PNIPAAm by UV-induced graft polymerization as described previously (Hu et al., 2002, 2004; Sugiura et al., 2008). Briefly, the flat PDMS plate was immersed for 1 min in acetone containing 10 wt.% benzophenone. The PDMS plate was immersed in a reaction mixture of NIPAAm monomer (10 wt.%), sodium periodate (0.5 mM) and benzyl alcohol (0.5 wt.%) dissolved in water. The PDMS plate was immediately exposed to UV light in the reaction mixture through a mask

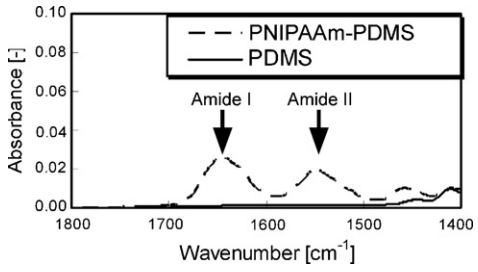

Fig. 2. ATR-FTIR spectra of an unmodified PDMS and a PNIPAAm–PDMS surface.

aligner (ES20AS, Nanometric Technology, Tokyo, Japan) for 5 min and washed with water to obtain the PNIPAAm-modified PDMS (PNIPAAm–PDMS).

A PDMS microchannel was fabricated by attaching a PDMS microchannel plate made via soft lithography to a flat PDMS plate by means of O_2 plasma bonding as described previously (Jo et al., 2000; Xia and Whitesides, 1998). For characterization of protein adsorption in the microchannel, the PDMS microchannel was modified with PNIPAAm by UV-induced graft polymerization. The UV-induced graft polymerization was carried out by means of the same procedure described above for the flat PDMS plate except that instead of immersing the PDMS plates described above, the solutions were introduced into the microchannel with syringe.

2.3. Surface characterization of PNIPAAm–PDMS

Surface modification of the PDMS plates with PNIPAAm was confirmed by attenuated total refection-Fourier-transform infrared (ATR-FTIR) spectroscopy using an FTIR spectrometer (FT/IR-680 Plus, JASCO, Tokyo, Japan) equipped with single reflection ATR accessories (MIRacle, PIKE Technologies, Madison, WI, USA).

The hydrophilicity of the PDMS and PNIPAAm–PDMS surfaces was evaluated by water-in-air static contact angle measurement with a contact angle meter (Drop Master 300, Kyowa Interface Science, Niiza, Japan). The samples were immersed in water at either 25 or 43 °C for 10 min prior to contact angle measurement. A water droplet was then placed on the surface with keeping temperature, and the water-in-air static contact angle was measured after 30 s. The contact angle was measured five times for each sample. Three different samples were prepared and tested for each experimental condition.

2.4. Modification of StAv with PNIPAAm

PNIPAAm terminated with a carboxyl group at one end (PNIPAAm-COOH) was prepared by chain-transfer polymerization using MPA as a chain-transfer reagent and AIBN as an initiator (Ding et al., 1996; Takei et al., 1993a). Briefly, 17.1 g of NIPAAm monomer, 0.08 g of MPA and 0.032 g of AIBN were dissolved in 76 mL of *N*,*N*-dimethylformamide (DMF). The solution was degassed by freezing and evacuating three times and then sealed. Polymerization was then carried out at 70 °C for 5 h, and the PNIPAAm-COOH product was collected by precipitation in diethyl ether.

The molecular weight of PNIPAAm-COOH was determined by gel permeation chromatography (GPC Linear LF-804, Showa Denko, Tokyo, Japan) in DMF containing 10 mM lithium bromide at 40 °C. A poly(ethylene glycol) standard was used (Polymer Laboratories Calibration Kit, GL Sciences, Tokyo, Japan; TSK Standard, Tosoh, Tokyo, Japan).

The terminal carboxyl group of PNIPAAm-COOH was activated with NHS using DCC as an activating reagent (Ding et al., 1996; Takei et al., 1993a). Briefly, 1.3 g (0.1 mmol) of PNIPAAm-COOH was dissolved in 13 mL of anhydrous methylene chloride and cooled to 0 °C. NHS (0.2 mmol) and DCC (0.2 mmol) were added under stirring, and stirring was continued for 4 h after raising the temperature to 20 °C. After reaction, the insoluble dicyclohexylurea byproduct was removed from the reaction mixture by filtration, and the polymer was isolated by precipitation in diethyl ether.

Activated PNIPAAm was reacted with StAv by the following procedure. The activated PNIPAAm (30 mg, 2.4 mmol) was dissolved in 43 μL of DMF. StAv (8.6 mg, 0.16 mmol) was dissolved in 430 μL of 20 mM phosphate-buffered saline (PBS, pH 7.6) at 4 °C. The two solutions were mixed and gently shaken at 4 °C overnight. The PNIPAAm–StAv conjugate was separated from unconjugated StAv and other water-soluble chemicals by thermally induced precipitation at 37 °C for 30 min and recovered by centrifugation at 17,400 × *g* at 37 °C for 10 min. The thermally induced precipitation process was repeated three times to remove any unreacted StAv. The protein content was determined by means of UV absorption measurement at 280 nm (A_{280}) with a spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE, USA). The prepared PNIPAAm–StAv was stored at 4 °C.

2.5. Characterization of PNIPAAm–StAv

To estimate the amount of the PNIPAAm present in the PNIPAAm–StAv, the polymer's primary amine groups were detected by a fluorometric assay as described previously (Stocks et al., 1986). Briefly, StAv and PNIPAAm–StAv were dissolved in 0.1 M sodium phosphate buffer (pH 8.0). Fluorescamine (3.8 mg) was dissolved in 10 mL of DMF. The two solutions were mixed and reacted at 4 °C for 10 min. The fluorescence of the mixed solution was measured on a fluorescence spectrometer (FP-750, JASCO) with an excitation wavelength of 388 nm and an emission wavelength of 485 nm. The excess fluorescamine in the mixed solution is rapidly destroyed by hydrolysis and does not contribute to the measured signal, whereas the fluorescence of the fluorescamine-conjugated polymer is stable for several hours (Oreilly and Lanza, 1995). The total titratable amine content in PNIPAAm–StAv was obtained from a calibration curve derived from the measured fluorescence intensity using native StAv at various concentrations.

The extent of temperature-change-induced precipitation (thermoprecipitation) for PNIPAAm–StAv, as well as its number of biotin binding sites was estimated with FITC–biotin. PNIPAAm–StAv (14 μM) was incubated with FITC–biotin at a 1:4 molar ratio in 250 μL of PBS at 4 °C for 2 h. The reaction mixture was incubated at 37 °C for 10 min and centrifuged at 17,400 × *g* at 37 °C for 30 min. The pellets were redissolved in PBS to achieve a total volume equal to the initial sample volume (250 μL). The amount of FITC–biotin in the supernatant left from centrifugation and in the redissolved pellet solution was estimated from fluorescence intensity measured with an excitation wavelength of 495 nm and an emission wavelength of 520 nm. The amount of StAv in the supernatant and in the redissolved pellet solution was estimated from measurements of A_{280}.

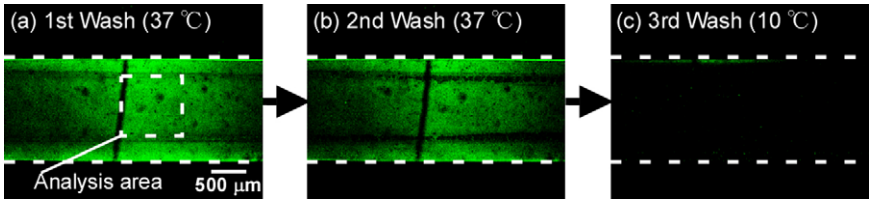

Fig. 3. CLSM images showing adsorption of PNIPAAm–StAv conjugated with FITC–biotin on a PNIPAAm–PDMS microchannel surface after washing at different temperatures. The CLSM images were obtained after washing with 0.5 mL PBS at 37 °C (a), after washing with another 1 mL PBS at 37 °C (b), and after washing with 1 mL PBS at 10 °C (c).

2.6. Thermoresponsive adsorption of PNIPAAm–StAv on PNIPAAm–PDMS

To demonstrate the thermoresponsive adsorption of PNIPAAm–StAv on PNIPAAm–PDMS, the adsorption of the modified protein on the modified surface was evaluated in terms of FITC–biotin conjugation and examined with a confocal laser scanning microscopy (CLSM; Fluoview FV300, Olympus). The amount of the adsorbed PNIPAAm–StAv conjugated with FITC–biotin was estimated as the fluorescence intensity calculated from images obtained above and below the LCST. The temperature of the microchannel was controlled by a water-circulation chamber on the CLSM. PNIPAAm–StAv (0.72 μM) was incubated with FITC–biotin at a 1:1 molar ratio in PBS at 4 °C for 1 h. The reaction mixture was introduced into the PNIPAAm–PDMS microchannel and incubated at 37 °C for 15 min. After washing the microchannel with 0.5 mL of preincubated PBS at 37 °C, a fluorescence image was obtained with the CLSM (excitation 488 nm; emission 518 nm). After 5 min of incubation, the microchannel was washed with 1 mL of PBS at 37 °C, and a fluorescence image was obtained. The microchannel was then cooled to 10 °C, and after 5 min of incubation at this temperature the microchannel was washed with 1 mL of PBS at 10 °C, and a fluorescence image was obtained. The fluorescence intensity in the determined analysis area was calculated by means of WinRoof image analysis software (Mitani Corporation, Fukui, Japan). For comparison, the same experiment was carried out with unmodified StAv as a substitute for PNIPAAm–StAv and with unmodified PDMS as a substitute for PNIPAAm–PDMS.

To demonstrate the thermoresponsive adsorption of biotinylated biomolecules on PNIPAAm–StAv, IgG was used as a model protein. FITC-IgG was labeled with biotin by means of Biotin Labeling Kit-NH_2 (Dojindo) with a protocol outlined in the manufacturer's product information. The biotinylated FITC-IgG (FITC-IgG-b) was recovered with PBS, and the amount of the IgG present in the solution was estimated by fluorescence intensity measurements with an excitation wavelength of 495 nm and an emission wavelength of 520 nm. FITC-IgG-b (33 μM) was incubated with PNIPAAm–StAv (130 μM) at a 1:4 molar ratio in 220 μL of PBS at 4 °C overnight. Thermoprecipitation was carried out by means of the procedure described above for purification of PNIPAAm–StAv, and the precipitate was redissolved in PBS for use in further experiments. The amount of FITC-IgG-b in the redissolved pellet solution was estimated by measurement of fluorescence intensity. The solution was diluted to a concentration of 0.75 μM FITC-IgG-b and was introduced into the PNIPAAm–PDMS microchannel. The thermoresponsive adsorption of FITC-IgG-b conjugated with PNIPAAm–StAv on PNIPAAm–PDMS was investigated with the CLSM by means of the procedure used for evaluating PNIPAAm–StAv conjugated with FITC–biotin. For comparison, the same experiment was carried out with FITC-IgG-b as a substitute for FITC-IgG-b conjugated with PNIPAAm–StAv and with PDMS as a substitute for PNIPAAm–PDMS.

3. Results and discussion

3.1. Characterization of the PNIPAAm–PDMS surface

Surface modification of the flat PDMS plate with PNIPAAm was examined by means of ATR-FTIR spectra and contact angle measurements. In the ATR-FTIR spectra of the PNIPAAm–PDMS (Fig. 2), peaks characteristic of PNIPAAm were observed at 1647 cm^{-1} (amide I, labeled in Fig. 2) and 1551 cm^{-1} (amide II). The water contact angle on the PNIPAAm–PDMS (average ± standard deviation) was 99.9 ± 7.8° at 43 °C and 69.7 ± 5.9° at 25 °C. In contrast, the water contact angle on the untreated PDMS surface was 113.2 ± 1.2° at 43 °C and 114.5 ± 1.1° at 25 °C. These results suggest that the PDMS surface was successfully modified with PNIPAAm by UV-induced graft polymerization.

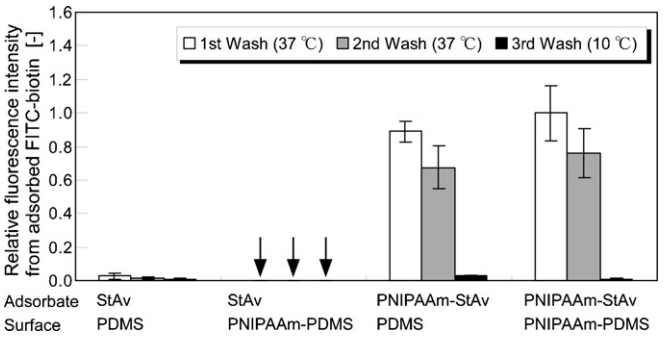

Fig. 4. Relative fluorescence intensity obtained from CLSM images for the adsorption of PNIPAAm–StAv or StAv conjugated with FITC–biotin on PNIPAAm–PDMS and PDMS microchannel surfaces after washing at different temperatures. Arrows pointing to the *x*-axis indicate undetectable adsorption. Error bars indicate the standard deviation of three replicate experiments.

3.2. Characterization of PNIPAAm–StAv

The number-averaged molecular weight of the synthesized PNIPAAm-COOH was determined to be 12,600 by GPC. PNIPAAm-COOH was activated by NHS with DCC and reacted with StAv. After recovery by thermoprecipitation, the yield of PNIPAAm–StAv was 10.5%. The number of reactive primary amine groups in the recovered PNIPAAm–StAv as determined by fluorescamine assay was only 46% of that in native StAv. Although we cannot determine how many of the 36 amine groups on each StAv molecule were reactive, the fluorescamine assay results indicate that more than half of the reactive primary amine groups were modified with PNIPAAm. The number of biotin binding sites in PNIPAAm–StAv was determined by thermoprecipitation of PNIPAAm–StAv reacted with FITC–biotin at a 1:4 molar ratio. The results showed that 77% of PNIPAAm–StAv and 19% of FITC–biotin in the initial reaction mixture precipitated at 37 °C, indicating that each PNIPAAm–StAv molecule contained an average of one biotin-binding site. These results suggest that StAv was successfully modified with PNIPAAm with keeping the biotin-binding site active.

3.3. Thermoresponsive adsorption of PNIPAAm–StAv on the PNIPAAm–PDMS microchannel surface

The adsorption of PNIPAAm–StAv conjugated with FITC–biotin on the PNIPAAm–PDMS microchannel surface was investigated with the CLSM, as shown in the CLSM images in Fig. 3. PNIPAAm–StAv conjugated with FITC–biotin was adsorbed on the PNIPAAm–PDMS surface at temperatures above the LCST (37 °C, Fig. 3a and b) and desorbed at temperatures below the LCST (10 °C, Fig. 3c). Above the LCST, hydrophobic interaction between dehydrated polymer chains of PNIPAAm–StAv and polymer chains on PNIPAAm–PDMS induced adsorption on the PNIPAAm–PDMS surface. Below the LCST, PNIPAAm chains both on StAv and on PDMS were hydrated, and the adsorbed PNIPAAm–StAv consequently desorbed from the microchannel surface.

To investigate the role of PNIPAAm chains on StAv molecule and that on PDMS surface, adsorption behavior was examined using unmodified StAv as a substitute for PNIPAAm–StAv and unmodified PDMS as a substitute for PNIPAAm–PDMS (Fig. 4). The amount of StAv conjugated with FITC–biotin adsorbed on the PDMS surface with the CLSM. PNIPAAm modification of StAv induced the adsorption of StAv on both the PNIPAAm–PDMS and unmodified PDMS

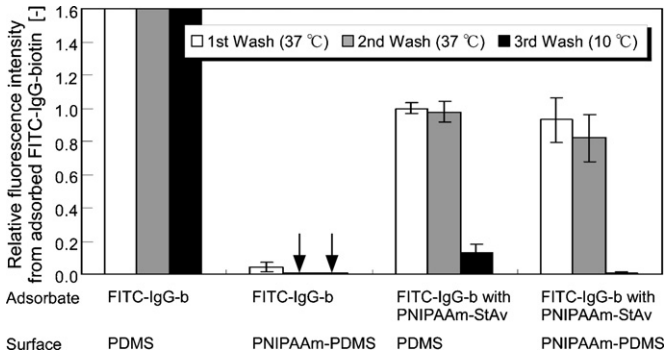

Fig. 5. Relative fluorescence intensity obtained from CLSM images for the adsorption of FITC-IgG-b conjugated with PNIPAAm–StAv or unmodified FITC-IgG-b on PNIPAAm–PDMS and PDMS microchannel surfaces after washing at different temperatures. Arrows pointing to the *x*-axis indicate undetectable adsorption. Error bars indicate the standard deviation of three replicate experiments.

microchannel surfaces at 37 °C. The adsorbed PNIPAAm–StAv was clearly desorbed at 10 °C from the both surfaces. In contrast, PNIPAAm modification of PDMS had little effect on the adsorption of PNIPAAm–StAv and StAv. The results indicate that the PNIPAAm modification of hydrophilic StAv plays an important role in the thermoresponsive adsorption. When both the protein and the surface were hydrophobic (as in the cases of PNIPAAm–StAv on PDMS at 37 °C and PNIPAAm–StAv on PNIPAAm–PDMS at 37 °C), the protein was adsorbed on the surfaces by hydrophobic interaction. When at least one of the components was hydrophilic (*i.e.*, unmodified, as in the cases of StAv on PDMS/PNIPAAm–PDMS at 37/10 °C and PNIPAAm–StAv on PDMS/PNIPAAm–PDMS at 10 °C), there was little hydrophobic interaction between the proteins and surfaces, and the protein was not adsorbed on the surface. Therefore, the modification of the hydrophobic PDMS surface with PNIPAAm did not appear to substantially contribute to the adsorption of the hydrophilic protein, StAv.

3.4. Thermoresponsive adsorption of FITC-IgG-b conjugated with PNIPAAm–StAv on the PNIPAAm–PDMS microchannel surface

We used IgG as a model protein to demonstrate the thermoresponsive adsorption of biotinylated biomolecules on microchannel surfaces. FITC-IgG-b conjugated with PNIPAAm–StAv was purified by thermoprecipitation and introduced into the PNIPAAm–PDMS microchannel, and the amount of adsorbed FITC-IgG-b was estimated from the resulting CLSM images. To investigate the role of PNIPAAm–StAv and of PNIPAAm on PDMS in the thermoresponsive adsorption of IgG, adsorption behavior was examined with FITC-IgG-b as a substitute for FITC-IgG-b conjugated with PNIPAAm–StAv and with PDMS as a substitute for PNIPAAm–PDMS (Fig. 5). When conjugated with PNIPAAm–StAv, FITC-IgG-b exhibited thermoresponsive adsorption on the PDMS and PNIPAAm–PDMS microchannel surfaces at 37 °C. This adsorption was induced by the hydrophobic interaction between the polymer chains on PNIPAAm–StAv and the hydrophobic surfaces (polymer chains on PNIPAAm–PDMS or PDMS itself). The PNIPAAm chains were hydrated at 10 °C, causing the adsorbed proteins to desorb from the microchannel surfaces. In the case of FITC-IgG-b adsorption on the bare PDMS surface, the fluorescence intensity was more than one order of magnitude higher than that under the other experimental conditions because hydrophobic interaction between the Fc group of IgG and the PDMS surface induced a high amount of physical adsorption. The physical adsorption of FITC-IgG-b on the PDMS surface was drastically reduced upon modification of the PDMS surface with PNIPAAm. This adsorption was most likely reduced because of steric hindrance induced by the PNIPAAm chains and because of the weaker hydrophobicity of PNIPAAm–PDMS relative to that of unmodified PDMS. This phenomenon also probably has contributed to the high-contrast thermoresponsive desorption of FITC-IgG-b conjugated with PNIPAAm–StAv: less than 1% of the adsorbed IgG-b was detected after the PNIPAAm–PDMS surface was washed at 10 °C.

The thermoresponsive physical property change of PNIPAAm is not induced by the chemical reaction but by the physical phase transition at the mild temperature condition around 37 °C. Therefore, the thermoresponsive physical property change is reversible phenomenon. The repeated thermoresponsive physical property changes were also reported on the thermoresponsive precipitation of PNIPAAm-modified proteins (Ding et al., 1996; Takei et al., 1993b) and on the thermoresponsive surface wettability change of PNIPAAm-modified surfaces (Liang et al., 1998, 2000). The technology presented in this study is based on the thermoresponsive property change of PNIPAAm–StAv and PNIPAAm–PDMS surface. Therefore, it is reasonable to consider that the PNIPAAm–PDMS surface is capable of repeated adsorption of PNIPAAm–StAv.

The technology presented in this study enabled high-contrast thermoresponsive protein adsorption on PDMS surfaces by using PNIPAAm–StAv. This system can be used for the adsorption of various biotinylated biomolecules through biotin–streptavidin chemistry. The high-contrast, controlled adsorption and desorption in the present technology can be applied to the regeneration of biosensor chips and to on-chip protein manipulation, including preconcentration and separation of soluble proteins.

4. Conclusions

The thermoresponsive adsorption of PNIPAAm–StAv on a PNIPAAm–PDMS microchannel surface was demonstrated. The thermoresponsive adsorption of biotinylated IgG conjugated with PNIPAAm–StAv was also demonstrated. PNIPAAm modification of StAv induced adsorption on the PDMS and PNIPAAm–PDMS surfaces by hydrophobic interaction. PNIPAAm modification of PDMS was revealed to be effective for reducing the extent of physical adsorption of IgG on PDMS surfaces. We also induced high-contrast thermoresponsive adsorption of FITC-IgG-b by modifying both StAv and PDMS with PNIPAAm. This controlled thermoresponsive protein adsorption will be useful for the development of future biosensors and microfluidic devices.

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