Thermoresponsive Copolymer Decorated Surface Enables Controlling the Adsorption of a Target Protein in Plasma

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Ⓢ Supporting Information

ABSTRACT: The control of protein/surface interactions by external stimuli is often required in bioapplications such as bioseparation and biosensors. Although regulation of protein adsorption has been achieved on the surfaces modified with stimuli-responsive polymers, controlled protein adsorption is still challenging for a target protein in a multiprotein system. The present study developed a concept of surface design for the controlled adsorption of a specific protein from plasma by combining a thermoresponsive polymer with an affinity ligand on the surface. In this regard, a polyurethane (PU) surface was modified with the copolymer of N-isopropylacrylamide (NIPAAm) and a ε-lysine-containing monomer (LysMA). ε-Lysine is a specific ligand for plasminogen that was used as the model "target protein" in this study. The PU-P(NIPAAm-co-Lys) surfaces exhibited distinct thermoresponsivity of plasminogen adsorption from plasma with a larger quantity adsorbed at 37 °C than at 23 °C. By contrast, the surfaces showed a low level of adsorption for other plasma proteins at both temperatures. In addition, plasminogen adsorbed on a PU-P(NIPAAm-co-Lys) surface could be partly desorbed by lowering the temperature, and the activity of plasminogen adsorbed was well preserved. We believe that the concept developed in this study can be extended to other proteins by combining PNIPAAm and specific ligands with affinities for the proteins of interest.

KEYWORDS: protein adsorption, thermoresponsive, specific ligand, selective, plasminogen
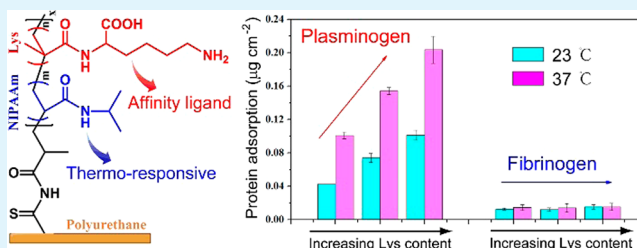
1. INTRODUCTION

Protein/surface interactions are thought to play an important role in determining the performance of a biomaterial when it is exposed to a biological environment. Control over the adsorption of proteins on the material surface is of crucial importance for a variety of biomedical applications.[1] It is suggested that nonspecific protein adsorption must be prevented for antifouling applications[2,3] while the adsorption of specific proteins with appropriate bioactivity would be beneficial for developing a specific biofunctionality.[4]

There are also many other cases such as protein purification and biosensors, in which protein adsorption is required to be regulated by external stimuli. To this end, stimuli-responsive surfaces, which can undergo dramatic physical or chemical changes upon specific environmental stimuli, have been employed to regulate protein adsorption by temperature,[5,6] pH/ionic strength,[7,8] or electric fields.[9] These surfaces are usually prepared by grafting environmentally sensitive polymers onto material surfaces, and act as switches for "turning on" and "turning off" the adsorption of proteins,[6,8] or for loading and releasing proteins.[9,10] In some cases, other hydrophobic or charged segments are incorporated by copolymerization to improve the binding capacity for proteins.[11,12]

However, stimuli-controlled adsorption of a target protein on the surfaces mentioned above can be hardly achieved because the attachment of proteins was mainly based on the nonspecific interactions. Introduction of specific ligands in the stimuli-responsive polymers may be one possible way for allowing the regulation of the adsorption of a target protein by external stimuli. Such a strategy was preliminarily attempted by Stenzel et al., in which a thermoresponsive surface grafted with the copolymer of N-isopropylacrylamide (NIPAAm) and N-acryloyl glucosamine (AGA) was prepared. The specific interactions of the carbohydrate moieties with concanavalin A could be regulated by changing temperature due to the thermoresponsive PNIPAAm segments.[13] However, nonspecific protein adsorption was not involved in this study.

The aim of the present study is to develop a concept of surface design for controlling the adsorption of a target protein from a complex system by changing temperature. The key of this concept is the combination of a thermoresponsive component, PNIPAAm, and a specific ligand for the target protein on the surface. For proof-of-concept, plasminogen and its affinity ligand, ε-lysine, were chosen as the models of the "target protein" and the "specific ligand", respectively. Plasminogen is the primary zymogen of the fibrinolytic pathway.[14] It contains two lysine binding sites (LBSs) that bind with high affinity to carboxy-terminal lysine residues

exposed on the surface of fibrin clots.[15,16] Therefore, ε-lysine, i.e., the lysine with both ε-NH_2 and −COOH free, has been utilized in some studies as an affinity ligand to capture plasminogen from plasma.[17] We have reported that the surfaces modified with polymers containing ε-lysine and nonfouling segments can bind plasminogen from plasma with a high degree of selectivity while reducing nonspecific protein adsorption significantly.[18−20]

Polyurethane (PU) was used as a platform to construct a surface containing both PNIPAAm and ε-lysine. This was achieved by the graft copolymerization of NIPAAm and a methacrylic monomer with a ε-lysine group (LysMA) on a vinyl-functionalized PU surface. Thermoresponsive though PNIPAAm is, it was found in some studies to be protein-repellant at the temperatures either above or below its lower critical solution temperature (LCST).[21,22] Therefore, the PU-P(NIPAAm-co-Lys) surface was expected to resist nonspecific protein adsorption while thermally regulating plasminogen adsorption in plasma. In other words, the surface would have the potential to regulate the adsorption of a target protein by changing temperature. Moreover, protein adsorption based on specific interactions would be beneficial for preserving protein's activity.

2. EXPERIMENTAL SECTION

2.1. Materials. Methacryloyl isothiocyanate (MI)[23] and 6-amino-2-(2-methacylamido)hexanoic acid (LysMA)[24] were synthesized as reported previously. Tecothane (TT-1095A) polyurethane was from Thermedics (Wilmington, MA) and was purified by Soxhlet extraction with toluene for 48 h. N-isopropylacrylamide (NIPAAm, Acros, 99%) was recrystallized from a toluene/hexane solution (50%, v/v) and dried under vacuum prior to use. 2,2′-Azoisobutyronitrile (AIBN) from Shanghai Qiangshun Chemical Reagent Co. was recrystallized from ethanol solution and dried under vacuum prior to use. N,N′-Dimethylformamide (DMF), triethylamine (TEA), and methanol were from the Shanghai Chemical Reagent Co. and purified before use. Trifluoroaceticacid (TFA) and 4-nitrobenzaldehyde were from Sigma-Aldrich Chemical Co. and used as received. Fibrinogen (plasminogen-free) was from Calbiochem (LaJolla, CA). Plasminogen was from Enzyme Research Laboratories (South Bend, IN). Na^{125}I was from Chengdu Gaotong Isotope Co., Ltd. Tissue plasminogen activator (tPA) was from Genentech (San Francisco, CA) and S-2251 was from GL Biochem Ltd., Shanghai.

2.2. Preparation of the PU Films. PU films were prepared by a solution-casting method. Briefly, 2.5 g of PU was dissolved in 50 mL of DMF. After the suspension was stirred for 6 h at 80 °C, the solution was casted into a glass dish and volatilized in vacuum at 80 °C for 24 h. The PU films were peeled off and punched into discs (~0.63 cm in diameter and ~0.2 mm thick).

2.3. Preparation of the Copolymer-Grafted PU Surfaces. A vinyl-functionalized PU surface (VPU) was prepared as reported previously.[20] Briefly, The PU discs were immersed in 25 mL of an acetonitrile solution containing 8 mmol MI and 2.5% (w/v) TEA. After the solution was stirred at 65 °C for 12 h, the vinyl-functionalized PU surface obtained was washed with acetonitrile and dried in a vacuum oven at 40 °C for 24 h.

P(NIPAAm-co-LysMA)-grafted PU surfaces were prepared by free radical copolymerization of NIPAAm and LysMA on VPU surfaces. NIPAAm or mixtures of NIPAAm and LysMA (with molar ratios of 30:1, 20:1, 10:1) were dissolved in methanol at a total monomer concentration of 1.75 mol/L. VPU films were immersed in the methanol solution and then azobisisobutyronitrile (AIBN) was added as the initiator (0.0167 mol/L). The solutions were degassed by bubbling with nitrogen for 20 min. The polymerization reaction was carried out under a nitrogen atmosphere at 65 °C for 12 h. After polymerization, the PU films were washed successively with methanol,

distilled water, and methanol and then dried in vacuum oven at 40 °C for 24 h.

Copolymers in solution were collected by precipitation. The compositions of the copolymers were analyzed using an INOVA 400 MHz nuclear magnetic resonance instrument (^{1}H NMR). The number-average molecular weights (Mn) and molecular weight distributions (M_w/M_n) of the copolymers were determined using a Waters 1515 gel permeation chromatograph (GPC). For the measurement of lower critical solution temperature (LCST), the copolymers were dissolved in PBS (pH = 7.4). The solutions were heated from 23 to 37 °C and the absorbance at 490 nm was read every increase of 2 °C. LCST was determined from the inflection point on the absorbance versus temperature curve.

2.4. Surfaces Characterization. Static contact angles were measured with a SL200C optical contact angle meter (USA Kino Industry Co., Ltd.) using the sessile-drop method. In brief, a film was placed on the sample stage equipped with a temperature-controlling element. After equilibration at the designated temperature for 20 min, 2 μL of distilled water with the same temperature was dropped on the film and the contact angle was read.

The lysine densities on the PU surfaces were determined using a previously reported method.[25] Typically, three discs of PU or modified PU films were immersed in anhydrous ethanol (10 mL) containing 4-nitrobenzaldehyde (10 mg) and acetic acid (0.008 mL) and kept under dark and nitrogen atmosphere at 50 °C for 3 h. The films were then washed and sonicated in absolute ethanol for 2 min. They were then immersed in water (3 mL) containing acetic acid (0.006 mL) and kept at 40 °C for 3 h. The 4-nitrobenzaldehyde liberated, equivalent to the surface amino content, was determined by measuring absorbance at 268.5 nm.

2.5. Protein Adsorption. Fibrinogen and HSA were labeled with ^{125}I using the iodine monochloride (ICl) method. The product was passed through an AG 1-X4 column to remove free iodide. Plasminogen was labeled with ^{125}I using the iodogen method, and was dialyzed overnight against five changes of PBS (pH 7.4) to remove unbound radioactive iodide. For studies of protein adsorption from plasma, labeled protein was added to citrated platelet-poor plasma at a concentration approximately 10% of the endogenous protein level.

The surfaces were equilibrated in PBS (pH 7.4) at 23 or 37 °C for 12 h prior to adsorption experiments. Then they were incubated with ^{125}I-protein-containing buffer or plasma for 3 h at 23 or 37 °C, rinsed three times (10 min each time) with PBS (pH 7.4), wicked onto filter paper, and transferred to clean tubes for radioactivity determination using a Wallac 2480 "Wizard 3"automatic gamma counter (PerkinElmer Life Sciences, Shelton, CT). The radioactivity of 10 μL of ^{125}I-protein-containing buffer or plasma was counted at the same time and the radioactivity per unit mass of protein was obtained for calibration. Protein adsorption was expressed as mass per unit surface area.

To investigate plasminogen desorption upon the change in temperature, PU-P(NIPAAm-co-LysMA) samples, after adsorption in plasma at 37 °C, were washed with PBS (pH 7.4) under 37 °C for three times (10 min each time) to remove the loosely bound proteins. Then the surfaces were incubated in PBS at 4 °C for 3 h, and plasminogen amounts remained on the surface were measured.

For protein adsorption measured by enzyme-linked immuno sorbent assay (ELISA), the surfaces were incubated in plasma for 3 h at 23 or 37 °C. Surfaces incubated in bovine serum albumin (BSA, 10 μg/mL in PBS) were used as a negative control. The surfaces were then washed with PBS and blocked with BSA (1% in PBS) for 1.5 h. After rinsed with PBS, the surfaces were incubated in primary antibodies against the protein of interest for 1 h and washed with PBS. Then the surfaces were incubated in alkaline phosphatase-conjugated secondary antibodies against the primary antibodies above and washed with PBS. The enzyme−substrate reaction was carried out in 0.1 M diethanolamine buffer (pH 9.8) containing 1 mM $MgCl_2$ at 37 °C with 1 mg/mL 4-nitrophenylphosphate disodium salt as the substrate. Finally, the absorbance at 405 nm was measured.

2.6. Chromogenic Substrate Assay for Plasminogen Activity. The enzymatic activity of plasminogen adsorbed on the copolymer-
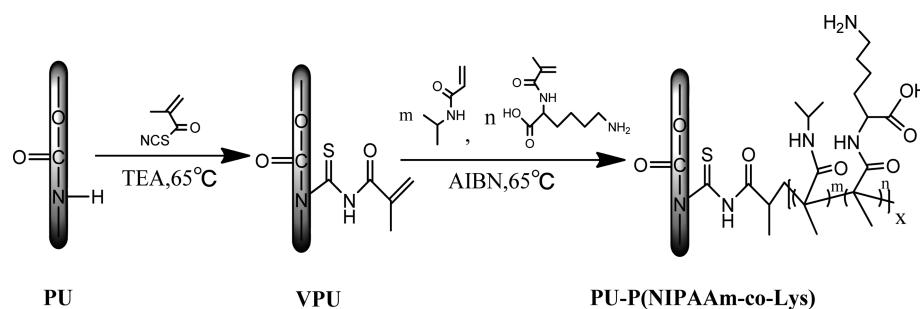
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Figure 1. Preparation of PU-P(NIPAAm-*co*-Lys) surfaces.

modified surfaces was determined using the specific chromogenic substrate for plasmin, S-2251. First, the copolymer-modified surfaces were incubated in radiolabeled plasminogen-containing plasma for 3 h at 37 °C and the quantities of plasminogen adsorbed were obtained by testing the radioactivity on the surfaces as described above. Free plasminogen (0.18 μg) and films with plasminogen adsorbed were incubated in t-PA solution (0.1 mg mL^{-1} in PBS) for 30 min at 37 °C and rinsed extensively with buffer. To the extent that plasminogen is adsorbed from plasma, this procedure provides surfaces bearing a layer of bound plasmin. Films were moved into new wells and 50 μL of 0.65 μM S-2251 and TBS (pH 7.5) were added to give a final volume of 250 μL. The optical density at 405 nm was measured over time at 37 °C using a microplate reader. The activity of plasminogen adsorbed on the surfaces was compared to that of free plasminogen.

3. RESULTS AND DISCUSSION

3.1. Free Radical Copolymerization on PU Surfaces.
The preparation of P(NIPAAm-*co*-Lys) is illustrated in Figure 1. First, a vinyl-functionalized PU surface (VPU) was obtained by reaction of MI with the −NH group on the PU. The graft polymerization of NIPAAm and LysMA on the VPU surface was then carried out by free radical polymerization using AIBN as the initiator. The copolymer grafted contained lysine residues with both ε-NH$_2$ and −COOH exposed, which have high binding affinities for plasminogen. The PNIPAAm component endowed the copolymer with thermoresponsivity.

Surface-grafted copolymers with varied compositions were obtained by using different feed ratios (NIPAAm: LysMA = 30:1, 20:1, 10:1, named as P1, P2, and P3, respectively). The copolymers formed simultaneously in solution were collected and characterized using ^{1}H NMR and GPC. As shown in Table 1, the lysine content in the copolymer increased with increasing

Table 1. Molecular Weights and Compositions of the Copolymers, And Lysine Densities on the Copolymer-Modified Surfaces

sample	f_{Lys}[a] (%)	F_{Lys}[b] (%)	$M_{n,GPC}$ (g mol^{-1})	M_W/M_n (GPC)	lysine density (nmol cm^{-2})
P-1	3.23	1.96	1.9×10^5	1.46	4.68
P-2	4.76	3.03	1.1×10^5	1.50	9.63
P-3	9.09	6.67	1.0×10^4	1.52	15.24

[a]Molar monomer feed ratio. [b]Molar composition of copolymers determined by ^{1}H NMR.

the feed fraction of LysMA in the polymerization, implying that the copolymer composition could be adjusted simply by changing the feed ratio. The Lys content in the copolymer is always lower than in the feed, presumably due to the large side group of LysMA that hinders the monomer from adding to the propagating chains. This is also the possible cause of decreased molecular weight with increasing LysMA content in the feed.

3.2. Surface Lysine Density.
The surface lysine density is an important parameter that determines the plasminogen binding capacity. As shown in Table 1, the lysine density increased as the copolymer lysine content increased. It should be noted that the molecular weight of surface-grafted polymers did not play a decisive role in the lysine contents on the surfaces. This is because the measurement of surface lysine density was based on the chemical reaction between 4-nitrobenzaldehyde and amino groups exposed on the surface, which might be of low efficiency at the sites close to the underlying substrate. That is to say, the values of surface lysine density listed in Table 1 reflect the lysine content in the upper layer of the polymer brush, and thus change with the copolymer lysine content (F_{Lys} in Table 1) following a similar trend. The upper layer of the polymer brush is also the main place where protein interacts with the surface. Therefore, the binding capacity for plasminogen was expected to be regulated by changing lysine content in this layer, which can be adjusted, in turn, by the feed ratio of monomers.

3.3. Wettability of Copolymer Modified PU Surfaces.
The static water contact angles measured at room temperature can indicate the variation of the surface wettability and thus the surface chemistry. As shown in Figure 2a, both PU and VPU are relatively hydrophobic with contact angles of 89° and 87°, respectively. Once grafted with P(NIPAAm-*co*-Lys), the surfaces behaved more hydrophilic with the contact angle around 62° independent of copolymer composition. This is because both PNIPAAm and lysine tend to be hydrated at room temperature.

It has been reported that the wettability of PNIPAAm-grafted surfaces may experience a temperature dependent change near the LCST of PNIPAAm.[26] PNIPAAm chains are hydrated with an extended conformation below its LCST but transform to a collapsed morphology when heated up to above the LCST due to dehydration. Water contact angles of the copolymer-modified surfaces were measured as a function of temperature, which may display the phase transition of the surface-grafted polymers. As shown in Figure 2b, the LCSTs of surface-grafted copolymers with the three kinds of composition are all around 28 °C. The difference of contact angles at 23 and 37 °C is ∼9°. The increase in the content of hydrophilic lysine moieties did not induce an increase in the LCST of the copolymer as expected. The exact reason is unknown. We speculate that crowding of the polymer chains in the polymer brush layer may facilitate the dehydration of PNIPAAm segments when temperature increased. Under this effect, the contribution from the little content of lysine (1.96% ∼ 6.67%) may be ignored.

3.4. Protein Adsorption.
3.4.1. Specificity to Plasminogen. Plasminogen contains five ternary loop structures
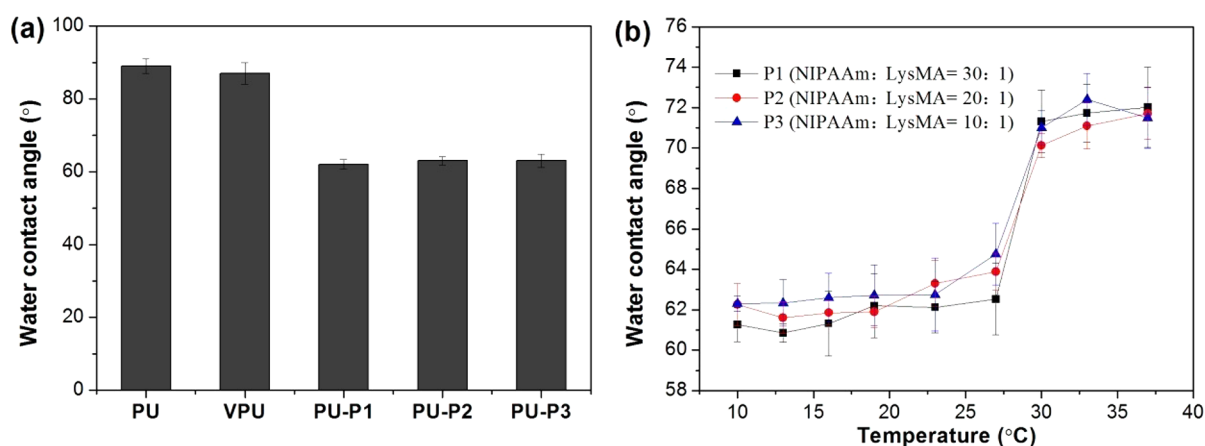
Figure 2. Water contact angles of PU and modified PU surfaces measured at 23 °C (a) and those of copolymer-modified surfaces as a function of temperature (b). Data = mean ± SD, n = 3.
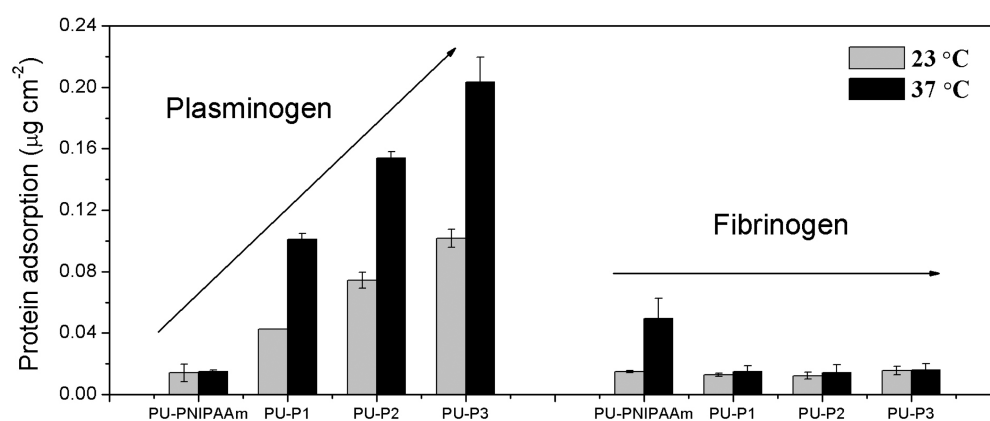

Figure 3. Amounts of plasminogen and fibrinogen adsorbed from plasma on the PNIPAAm- and P(NIPAAm-*co*-Lys)-modified PU surfaces at 23 and 37 °C. PU-P(NIPAAm-*co*-Lys) surfaces exhibited distinct thermo-responsivity of plasminogen uptake but excellent resistance to fibrinogen adsorption. Data = mean ± SD, n = 3.

known as "kringles", two of which contain a lysine binding site (LBS). It is through the LBSs that plasminogen binds with high affinity to the surface-bound ε-lysine.[27−29] As seen in Figure 3, the PU-PNIPAAm surface does not possess affinity binding sites for plasminogen, and thus showed very low adsorption (∼14 ng cm^{-2}), regardless of temperature. Copolymer-modified surfaces showed a higher level of plasminogen adsorption than the PU-PNIPAAm surface, due to specific binding of plasminogen to ε-lysine residues. As expected, the quantity adsorbed increased with surface lysine density. The amount of plasminogen adsorbed on the surface with the highest lysine content at 37 °C is comparable to that adsorbed on a P(HEMA-*co*-Lys)-grafted PU surface with similar lysine content reported previously.[30]

3.4.2. Resistance to Nonspecific Protein Adsorption. Fibrinogen is abundant in plasma and highly surface-adhesive. Therefore, fibrinogen adsorption from plasma was tested to provide indications of nonspecific/nontarget protein adsorption. As shown in Figure 3, fibrinogen adsorption exhibited a little thermoresponsivity on the PU-PNIPAAm surface with a larger amount adsorbed at 37 °C than at 23 °C, and an extremely low level on all the copolymer-modified surfaces. ELISA results showed that the amount of human plasma albumin (HSA) adsorbed on the P(NIPAAm-*co*-Lys)-modified PU surface was similar to that of fibrinogen, and IgG adsorbed was even less than fibrinogen. Therefore, the PU-P(NIPAAm-

co-Lys) surface has resistance against plasma proteins other than plasminogen. It has been reported that PNIPAAm-modified surfaces have good resistance to nonspecific protein adsorption with an unremarkable difference in the quantity of protein adsorbed at temperatures below and above the LCST.[21,31] Therefore, the low level of fibrinogen adsorption on the PU-P(NIPAAm-*co*-Lys) surfaces is partially attributed to the nonfouling nature of PNIPAAm components. Another contribution is from the preferential surface uptake of plasminogen, the most competitive protein in plasma for binding ε-lysine; other nonspecific proteins are thus suppressed or displaced by plasminogen after attaching on the surfaces.[32] This is exactly the mechanism of the Vroman effect.[33] It should be noted that if plasminogen is adsorbed on PU-P(NIPAAm-*co*-Lys) surfaces mainly through hydrophobic or electrostatic interactions, then fibrinogen should have shown a similar level of adsorption as plasminogen. Therefore, different binding capacities for plasminogen and fibrinogen at 37 °C demonstrated that plasminogen was associated through affinity recognition by ε-lysine rather than other nonspecific interactions with the surface.

3.4.3. Thermoresponsivity of Plasminogen Adsorption. According to the LCST of surface-grafted copolymers confirmed by contact angle measurements, protein adsorption may be regulated between 23 and 37 °C. As seen in Figure 3, it is particularly interesting that much more plasminogen was

adsorbed at 37 °C than at 23 °C on a given copolymer-modified surface. At a temperature below the LCST, the predominantly hydrogen bonding between PNIPAAm components and water molecules contributed to a hydration layer and extended flexible polymer chains, which prevented plasminogen from approaching to the ε-lysine residues. At a temperature above the LCST, the destruction of the hydration layer facilitates the binding of plasminogen by ε-lysine. In contrast to plasminogen adsorption, fibrinogen adsorption showed an extremely low level on all the copolymer-modified surfaces and no significant difference at 23 and 37 °C.

The PU-P(NIPAAm-co-Lys) surfaces exhibited distinct thermoresponsivity of plasminogen adsorption but good repellency to other plasma proteins suggesting the ability of selective control of plasminogen adsorption by thermostimuli. The extent of plasminogen adsorption that can be thermally controlled could be regulated by changing the surface lysine content. As shown in Figure 4, the difference of plasminogen
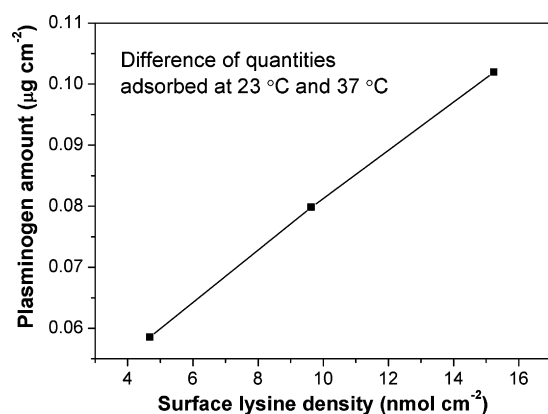

Figure 4. Difference of the amounts of plasminogen adsorbed from plasma on the PU-P(NIPAAm-co-Lys) surfaces at 23 and 37 °C as a function of surface lysine density.

amounts adsorbed at 23 and 37 °C is linearly correlated with the surface lysine density. This suggests that the thermoresponsivity of plasminogen adsorption can be adjusted by changing the composition of the copolymer grafted. In addition, 41% of the total amount of plasminogen adsorbed on PU-P(NIPAAm-co-Lys) surface (P3) could be desorbed by lowering temperature as shown in Figure 5. For the amount of
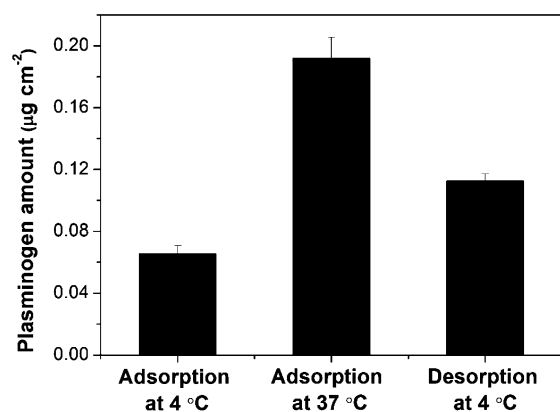

Figure 5. Plasminogen adsorption on PU-P(NIPAAm-co-Lys) surface (P3) at 4 and 37 °C for 3 h, and desorption at 4 °C for 3 h. Data = mean ± SD, n = 3.

plasminogen adsorbed by elevating the temperature from 4 to 37 °C, 62% can be desorbed. It means that the association of the target protein is partly reversible, which endows the surface with potential application in protein purification. However, further optimization is needed to meet the requirement of a specific application.

3.4.4. Plasminogen Activity. Stimuli-control over the adsorption of a target protein in a complicated system is the most notable advantage over the stimuli-responsive surfaces previously developed for regulating protein adsorption. This is attributed to the incorporation of highly specific ligands that can bind target proteins through specific interactions. It is worth mentioning that besides achieving selectivity for the target protein, specific interaction is always in favor of maintaining the protein's activity. In this regard, we investigated the activity of plasminogen adsorbed at 37 °C. This involves the conversion of plasminogen to plasmin by tPA and the catalytic activity of plasmin. As shown in Figure 6, the activity of surface-
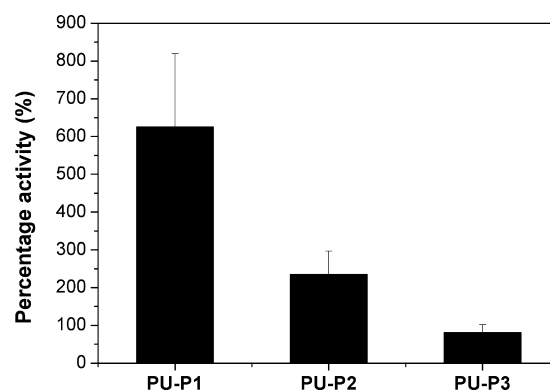

Figure 6. Activity of plasminogen adsorbed on the PU-P(NIPAAm-co-Lys) surfaces at 37 °C and activated by tPA. Hundred percent corresponds to the specific activity of free (not adsorbed) plasminogen. Data = mean ± SD, n = 3.

bound plasminogen decreased as the surface lysine content increased. Nevertheless, the surface with even the least plasminogen activity could present more than 80% activity of the free plasminogen. It is striking that the activities of plasminogen adsorbed on P1 and P2 surfaces are even higher than the activity of free plasminogen. It has been reported that binding with 6-aminohexanoic acid (analogous to ε-lysine) induces a conformational change in plasminogen from a closed form to an open form. This open form is more flexible and extended, which facilitates the binding of its activator and thus is more readily activated than the closed form.[34] This may explain why the plasminogen associated with ε-lysine showed improved activity as compared with free plasminogen. When the surface lysine content increased, plasminogen became crowded on the surface. On the PU-P3 surface, for example, the amount of plasminogen adsorbed was ~200 ng/cm², which was close to the theoretical value for a densely packed plasminogen monolayer (~256 ng/cm² based on a Rg of 39 Å for native plasminogen). Adsorption in such a crowded form may diminish the advantage of the open form of plasminogen, thus showing lower enzymatic activity.

4. CONCLUSION

The present study developed a concept of surface design for the stimuli-regulation of specific protein adsorption from plasma by

combining a stimuli-responsive polymer with a specific ligand on the surface. The surfaces containing ε-lysine and PNIPAAm were prepared by the graft copolymerization of NIPAAm and LysMA on vinyl-functionalized PU surfaces. The composition of the copolymer grafts (P(NIPAAm-co-Lys)) as well as the surface lysine density could be adjusted by varying the monomer feed ratio. The change of water contact angle with increasing temperatures for a P(NIPAAm-co-Lys)-grafted surface showed that its phase transition was at around 28 °C, independent of the polymer composition investigated. The PU-P(NIPAAm-co-Lys) surfaces exhibited distinct thermorespon-sivity of plasminogen adsorption in plasma with larger quantity adsorbed at 37 °C than at 23 °C, whereas the adsorption of other plasma proteins showed extremely low levels at both temperatures. The attachment of plasminogen on the copolymer-modified surfaces was mainly based on the specific interactions with ε-lysine. The difference in the quantity of plasminogen adsorbed at the two temperatures was linearly correlated with the surface lysine density and plasminogen adsorbed on PU-P(NIPAAm-co-Lys) surface could be desorbed to some extent by lowering temperature. The activity of plasminogen adsorbed on the PU-P(NIPAAm-co-Lys) surfaces was not only well preserved but even higher than the activity of free plasminogen.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Adsorption of fibrinogen, HSA, and IgG from plasma on PU and PU-P(NIPAAm-co-Lys) surfaces measured by ELISA; raw data of specific activity of free plasminogen and the plasminogen adsorbed on PU-P(NIPAAm-co-Lys) surfaces. 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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