Macromolecular Research, Vol. 14, No. 1, pp 73-80 (2006)

Surface Modification of Polyurethane Using Sulfonated PEG Grafted Polyrotaxane for Improved Biocompatibility

Hyung Dal Park†, Jin Woo Bae, and Ki Dong Park*

Dept. of Molecular Science and Technology, Ajou University, Suwon 443-749, Korea

Tooru Ooya and Nobuhiko Yui

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan

Jun-Hyeog Jang

Dept. of Biochemistry, College of Medicine, Inha University, Incheon 402-751, Korea

Dong Keun Han

Biomaterials Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea

Jung-Woog Shin

Dept. of Biomedical Engineering, Inje University, Gimhae 621-749, Korea

Received October 6, 2005; Revised December 12, 2005

Abstract: Sulfonated poly(ethylene glycol) (PEG-SO_3) grafted polyrotaxanes (PRx-PEG-SO_3) were prepared in order to utilize the unique properties of PEG-SO_3 and the supramolecular structure of PRx, in which PEG-SO_3 grafted α-cyclodextrins (α-CDs) were threaded onto PEG segments in a PEG-*b*-poly(propylene glycol) (PPG)-*b*-PEG triblock copolymer (Pluronic) chain capped with bulky end groups. Some of the PRx-PEG-SO_3 demonstrated a higher anticoagulant activity in case of PRx-PEG-SO_3 (P105), and compared with the control they showed a lower fibrinogen adsorption in PRx-PEG-SO_3 (F68) and a higher binding affinity with fibroblast growth factor. The obtained results suggested that polyrotaxane incorporated with PEG-SO_3 may be applicable to the surface modification of clinically used polymers, especially for blood/cell compatible medical devices.

Keywords: polyrotaxane, sulfonated polyethylene glycol, anticoagulant activity, protein adsorption, biocompatible.

Introduction

Biocompatibility of biomaterials is believed to be strongly influenced by a layer of host proteins and cells spontaneously adsorbed to the surfaces upon their implantation.[1-3] These *in vivo* responses of biomaterials have been extensively studied for their predictable routes after implantation. There has been a number of attempts to create a novel surface that reduces the adverse effects of biological interaction with the materials, without changing its bulk property. Especially, many researchers have been focused on the initial surface phenomena such as the protein adsorption and platelet adhesion among a variety of biological responses for the evaluation of biocompatibility. One of the most commonly used approaches has been the surface modification with various macromolecules, such as albumin, heparin, thrombomodulin, hirudin, and PEG.[4-7] PEG has been frequently used as a surface-modifying agent because PEG has been known to protect the underlying substrate by nonspecifically repelling proteins and cells.[8-10] Also, sulfonated PEG (PEG-SO_3)-modified surfaces have been demonstrated as the enhanced blood compatibility, biostability, and calcification-resistance.[11-13] These results suggested that 1) the unique properties of PEG chains such as low interfacial free energy, nonadhesive property, and highly dynamic mobility and 2) the pendant negatively charged sulfonate group with anticoagulant activity like heparin[14] and with the electrical repul-

*Corresponding Author. E-mail: kdp@ajou.ac.kr

†Current address; Phamaceutical Exam. Div., Chemistry & Biotechnology Exam. Bureau, Korean Intellectual Property Office (KIPO), Government Complex-Daejeon, Daejeon 302-701, Korea

sion on the blood components contribute to these phenomena.

Another promising characteristic of the sulfonated PEG is the binding affinity with growth factors.[15] The heparin-like sulfonate groups are expected to enhance the binding affinity with growth factors since heparin is known to modulate the bioactivity of heparin-binding growth factor and cell-related cytokines through different mechanism. Basic fibroblast growth factor (bFGF), a 17 kDa polypeptide, is a potent modulator of cell proliferation, motility, differentiation, and survival, as well as plays an important role in tissue regeneration.

In the modification by PEG-SO_3, the chemically modifiable reaction site is limited to only the terminal groups of substrates, which may lead to restricted effect of PEG-SO_3. Thus, new surface-modifying biomaterials with many hydroxyl groups for chemical modification are required in order to enhance the effect of PEG-SO_3. Supramolecular chemistry has been getting a fascinating new field of macromolecular architecture mimicking supramolecular systems in nature. Inclusion complexes consisting of cyclic molecules and polymeric chains have been prepared as novel molecular assemblies. Macromolecular recognition by cyclodextrins (CDs) with PEG have been investigated as a family of polyrotaxanes (PRx)[16] and it has been reported that the size of CD cavity and the cross-section area of the polymer chain are closely related to the interaction between CD cavity and the polymer chain. For example, α-CDs are known to form an inclusion complex not with PPG but with PEG.[17] The significant characteristics of polyrotaxanes involve 1) supramolecular structure and 2) chemical modification of cyclic molecules with many hydroxyl groups (typically, CDs). The former shows that the cyclic molecules behave as a molecular plaster to untie the random coil of the polymer chains and the latter shows that a lot of hydroxyl groups of the threaded CDs can be chemically modified to alter the physicochemical properties.[18]

In previous study, we have prepared PRx-SO_3, in which sulfonated α-CDs were threaded onto the PEG chains of Pluronic capped with bulky end groups. α-CD and Pluronic were selected for the main components of the PRx because α-CDs can be threaded onto the PEG segment of Pluronic and PPG segment of Pluronic can physically be immobilized on conventional polymer surface.[19] The PRx-SO_3 has demonstrated the enhanced anticoagulant activity in solution[20] and the improved biocompatibility on modified polymer surface,[21] suggesting that both the PRx structure and the sulfonated groups contribute to these phenomena.

In this study, PEG-SO_3 grafted PRx (PRx-PEG-SO_3) has been prepared in order to utilize the unique properties of PEG-SO_3 in combination with the supramolecular structure of PRx. The supramolecular structure and the chemical composition of the PRx-PEG-SO_3 have been characterized by FTIR ^{1}H-NMR and ^{13}C-NMR. The surface and bulk properties of PRx-PEG-SO_3 modified polyurethane surface have been characterized by contact angle measurements, electron spectroscopy for chemical analysis (ESCA), and stability measurements. The anticoagulant activity, plasma protein adsorption behaviors, the binding affinity to basic fibroblast growth factor have been evaluated *in vitro*.

Experimental

Materials. The PEG-*b*-PPG-*b*-PEG triblock copolymers (Pluronic: P105 and F68) were kindly supplied from Asahi Denka Kogyo K.K., Tokyo, Japan and BASF Co., USA, respectively (Table I). α-CD, benzyloxycarbonyl(Z)-L-phenylalanine (Z-L-Phe), *N,N'*-carbonyldiimidazole (CDI), ethylenediamine, 1,3-propane sultone, and calcium chloride were purchased from Wako Pure Chemical Ind., Osaka, Japan. *N*-Hydroxysuccinimide (HOSu) was purchased from Peptide Institute Co., Osaka, Japan. PEGs (M_n=300, 600, 1,000), heparin from Porcine Intestinal Mucosa, Rabbit serum albumin (RSA), and rabbit plasma fibrinogen (RPF) were purchased from the Sigma Chemical Co., USA. Standard Human Plasma and Pathromtin®SL were purchased from Dade Behring, Marburg, Germany. Polyurethane (Pellethane®) was obtained from Dow Chemical Co., Midland, MI, USA. The bicinchoninic acid (BCA) protein assay reagents were purchased from Pierce, Rockford, IL, USA. Unless otherwise specified, all chemicals were purchased from Aldrich and Sigma Chemical.

Preparation of PRx-PEG-SO_3. PRx was prepared by the method reported previously.[20] Briefly, PRx (**1**) was synthesized by capping reaction between the inclusion complex consisting of α-CDs and the Pluronics (P105 and F68, M_w of PEG–PPG–PEG: 1625-3250-1625 and 3250-1625-3250), and Z-L-Phe-succinimide ester (Z-L-Phe-OSu). The average number of α-CDs in the polyrotaxanes was calculated to be 15 and 26 by peak integrations of 4.79 ppm (C^{1}H of α-CD) and 3.51 ppm (CH_2 of PEG), respectively.

A lot of hydroxyl groups of α-CDs in the PRx were activated by carbonyldiimadazole (CDI) for the reaction with the remained amino-terminal groups of PEG-SO_3. CDI (2 equiv. to the -OH mole of α-CDs in the PRx) in dry dimethylsulfoxide (DMSO) was added to the PRx in DMSO and stirred for 6 h at room temperature under a nitrogen atmosphere. The reaction mixture was precipitated in excess diethyl ether and dried *in vacuo*, to give CDI-activated PRx (**2**).

To prepare NH_2-PEG-SO_3, 1,3-propane sultone (1/2 equiv. to the mole of the terminal amino groups of NH_2-PEG-NH_2) in tetrahydrofuran (THF) was added slowly to the diamino-terminated PEGs (NH_2-PEG-NH_2, AT-PEG) solution in THF and was stirred at 50 °C for 5 h. The resultant products were precipitated in cold THF, filtered, and dried *in vacuo* to give NH_2-PEG-SO_3. NH_2-PEG-SO_3 (8 equiv. to the OH mole of PRx) in dry DMSO was slowly added to the DMSO solution of CDI-activated PRx (**2**) and was stirred for 12 h

Table I. Synthetic Results of PRx-PEG-SO_3

Sample	Code	No. of α-CD	Yield (%)	DS[d]	No. of SO_3[e]
P105 series	(M_n of PEG-PPG-PEG)				
P105	(1625-3250-1625)	15[a]	-	-	-
PRx	15α/P105-PHE-Z	37[b]	25	-	-
PRx-S	20S-9α/P105-PHE-Z	45[c]	30	2.3	20
PRx-E0.3-S	121S-E0.3-15α/P105-PHE-Z		15	8.1	121
PRx-E0.6-S	108S-E0.6-15α/P105-PHE-Z		16	7.2	108
PRx-E1.0-S	93S-E1.0-15α/P105-PHE-Z		22	6.2	93
F68 series					
F68	(3360-1680-3360)	26[a]	-	-	-
PRx	26α/F68-PHE-Z	76[b]	29	-	-
PRx-S	50S-17α/F68-PHE-Z	34[c]	34	2.9	50
PRx-E0.3-S	195S-E0.3-26α/F68-PHE-Z		20	7.6	195
PRx-E0.6-S	179S-E0.6-26α/F68-PHE-Z		22	6.9	179
PRx-E1.0-S	159S-E1.0-26α/F68-PHE-Z		25	6.1	159

[a]Number of included α-CDs in PRx by ^{1}H-NMR spectra. [b]Number of calculated α-CDs by stoichiometric ratio (α-CD:ethylene glycol=1:2). [c]Portion of PEG segment included by α-CDs [= (a/b)×100 (%)]. [d]Degree of substitution (DS) per one α-CD by peak areas from ^{1}H-NMR (one α-CD has 18 hydroxyl groups). [e]Number of SO_3^- groups (= Number of α-CDs×DS).

at room temperature under a nitrogen atmosphere. The obtained solutions were evaporated to remove the solvent, dialyzed (MWCO=3,500) against distilled water, and finally lyophilized to give PRx-PEG-SO_3 (**3**) (Figure 1).

PU Modification Using PRx-PEG-SO_3. The purified PU was dissolved in dimethylacetamide (DMAc) to obtain a 12 wt% solution and the PRx-PEG-SO_3 with three kinds of PEGs (M_n of PEG=300, 600, 1,000) were added to the PU solutions at a concentration of 2.0 wt% based on the dry PU. Films were prepared by the solvent evaporation method on clean glass. The solvent was slowly evaporated at 35 °C for 6 d in a desiccator cabinet and followed by drying *in vacuo* at 50 °C for 3 d. Each of the prepared PUs was designated as PU/PRx-PEG (0.3,0.6,1.0k)-SO_3 or PU/PRx-E (0.3,0.6,1.0)-S (P105 or F68), respectively.

For the characterization of the modified PU surfaces, the wettability and the chemical compositions of the surface regions were determined by the measurement of the static contact angle and ESCA (Mg K_α source, ESCALAB MK II, V.G. Scientific Co., UK). The swelling property was examined by measuring the water absorption content: Water absorption (%)=$(W_s - W_d)\times 100/W_d$; where W_s and W_d are the weights of the swollen and dried samples, respectively. The stability of the incorporated PRx-PEG-SO_3 was estimated by a change in the weight after immersion into water: Extraction rate (%)=$(W_d - W_{d,final})\times 100/W_d$; where $W_{d,final}$ is the weight of fully dried samples after immersion for 7 d.

Determination of Anticoagulant Activity of PRx Derivatives. In conjunction with incubated plasma, Pathromtin®SL enables the individual factors of the intrinsic coagulation system to be quantified and permits diagnosis of hemophilia. It can be also used for monitoring heparin therapy.[22]

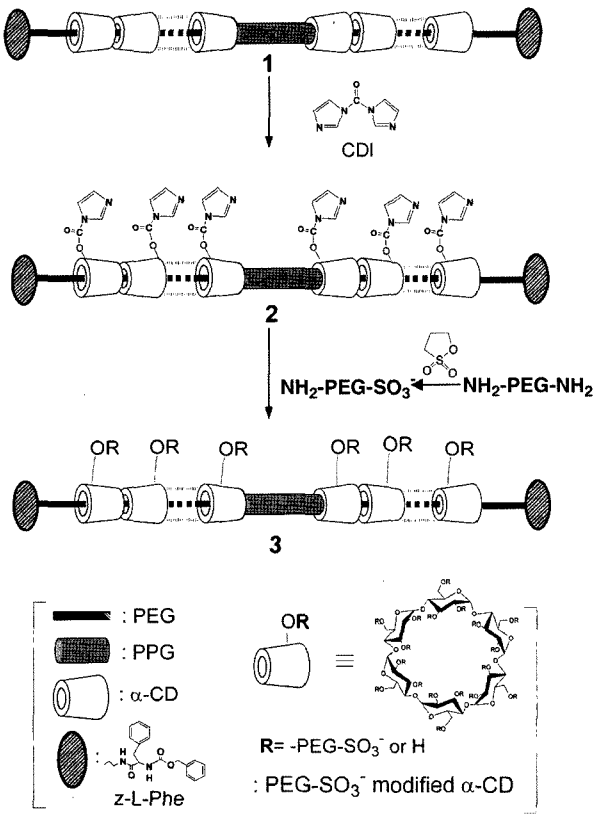

Figure 1. Synthetic procedure of PRx-PEG-SO_3.

Calcium ions of calcium chloride trigger the coagulation process; the time to formation of a fibrin clot is measured. Briefly, citrated standard plasma (50 μL) and Pathromtin®SL (50 μL) were pipetted into a test tube pre-warmed to 37 °C, and it was incubated at a constant time at 37 °C. Then, the aqueous solution of the PRx derivatives (PRx-SO_3 and PRx-PEG-SO_3) (50 μL, 0.1 wt%) was injected into the test tube. On adding the calcium chloride solution (50 μL, 0.025 M) at 37 °C, the timer on a coagulation analyzer (SYSMEX CA 50, Kobe, Japan) starts to measure the clotting time.

Plasma Protein Adsorption. As a preliminary study, the protein adsorption profile on modified surfaces was evaluated *in vitro*. The extent of albumin and fibrinogen adsorbed on the substrates was analyzed by BCA protein assay.[23] After equilibrium with phosphate buffered solution (0.1 M PBS, pH 7.4), each of the PUs (1×1 cm^2) was incubated in syringes containing 3 mL of RSA (460 μg mL^{-1}) and RPF (40 μg mL^{-1}) at 37 °C for 60 min. Each of the PUs was then rinsed with PBS and incubated with 2 mL of PBS containing 1.0 wt% sodium dodecylsulfate (SDS) for 1 h to measure the amount of irreversibly adsorbed proteins on the surfaces. The BCA working reagent was added to the protein containing solutions, followed by incubation at 60 °C for 60 min. After cooling to room temperature, the protein concentration was measured by the absorbance at 562 nm on a microplate reader (BioRad-550, Osaka, Japan).

bFGF Adhesion Assay. bFGF is well known to include a heparin-binding domain. For the construction of expression plasmids and purification, bFGF cDNAs was in-frame ligated into the multiple cloning sites of pBAD-His-B (Invitrogen) with the C-terminal 6X His tag. The bFGF containing poly-His tag were expressed and purified using a Ni^{2+} affinity column according to the manufacturer's protocol (Invitrogen).

The mixture solution of PRx derivatives and PU in DMAc were spin-coated on glass plates. Coated samples were placed in the bottoms of the 24-wells culture dish and coated with recombinant His-tagged bFGF for 1 d at 4 °C. After coating, samples were exposed to a horseradish peroxidase (HRP)-conjugated His antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at 37 °C. A colorimetric substrate for the HRP was then added (Pierce, Rockford, IL), and the absorbance at 450 nm was read.

Results and Discussion

Preparation and Characterization of PRx-PEG-SO_3. Characterization of NH_2-PEG-SO_3 was performed by FTIR and ^{1}H-NMR. As shown in Figure 2, the asymmetric and symmetric stretching peaks of SO_3 at the NH_2-PEG1.0k-SO_3 were observed around 1200 and 1040 cm^{-1} with no significant changes of N-H bond stretching absorptions in the 3300-3500 cm^{-1} range of the IR spectrum. Also, the new broad three proton peaks of sulfopropyl group (-$CH_2CH_2CH_2SO_3^-$) from 1,3-propane sultone were observed at ^{1}H-NMR spectra.

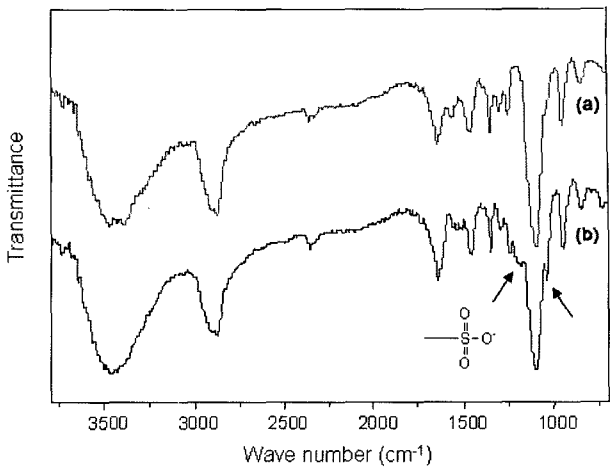

Figure 2. IR spectra of the amino-terminated PEG (AT-PEG1.0k) (a) and the one-terminal sulfonated PEG (NH_2-PEG1.0k-SO_3) (b).

The IR and ^{1}H-NMR spectra of NH_2-PEG (0.3,0.6k)-SO_3 were also similar to those of NH_2-PEG1.0k-SO_3. These results suggest that NH_2-PEG-SO_3 is a statistically dominant product after the sulfonation reaction by the stoichiometric molar ratio.

In the ^{1}H-NMR spectra of PRx-PEG(1.0k)-SO_3 (Figure 3), the signals of protons of glucose rings in α-CD were shielded by a strong signal from PEG protons of the grafted PEG-SO_3. However, the broad proton peaks of a sulfopropyl group (-$C^{\alpha}H_2C^{\beta}H_2C^{\gamma}H_2SO_3$) from 1,3-propane sultone were observed around 1.7-3.1 ppm (α: 2.5-2.7, β: 1.7-1.9, γ: 2.9-3.1 ppm) in addition to the typical peaks of the PRx.

From the ^{13}C-NMR spectra of PRx-PEG(1.0k)-SO_3, some of new carbon peaks including the signals of sulfopropyl group (-$\underline{C}^{\alpha}H_2\underline{C}^{\beta}H_2\underline{C}^{\gamma}H_2SO_3$: α, 26-27; β, 22-23; γ, 49-50 ppm) and PEG from the grafted PEG-SO_3 were shown. But, it was difficult to characterize all the carbon signals in PRx-PEG1.0k-SO_3 completely, which is likely to be associated with the statistical substitution positions of the -OH groups (one α-CD has 18 hydroxyl groups) and a crowding effect of the induced PEG substituents. In case of the PRx-PEG(0.3,0.6k)-SO_3, the ^{1}H- and ^{13}C-NMR spectra were also similar to that of PRx-PEG(1.0k)-SO_3 (data not shown).

The synthetic results of PRx-PEGs-SO_3 are summarized in Table I. The number of included α-CDs in PRx-PEGs-SO_3 was difficult to be estimated by the ^{1}H-NMR spectra, because the protons of PEG segments in PRx were broadened by a strong signal from PEG protons of the grafted PEG-SO_3. Thus, the number of α-CDs in PRx-PEG-SO_3 was considered as that of PRx before the chemical modification. With increasing the PEG molecular weight of the grafted PEG-SO_3, the DS (degree of substitution) and the number of SO_3 groups slightly decreased. These results indicate that the intramolecular hydrogen bonding between α-CDs and the higher PEG chain length of the PEG-SO_3 affect the reaction site as steric hindrance.

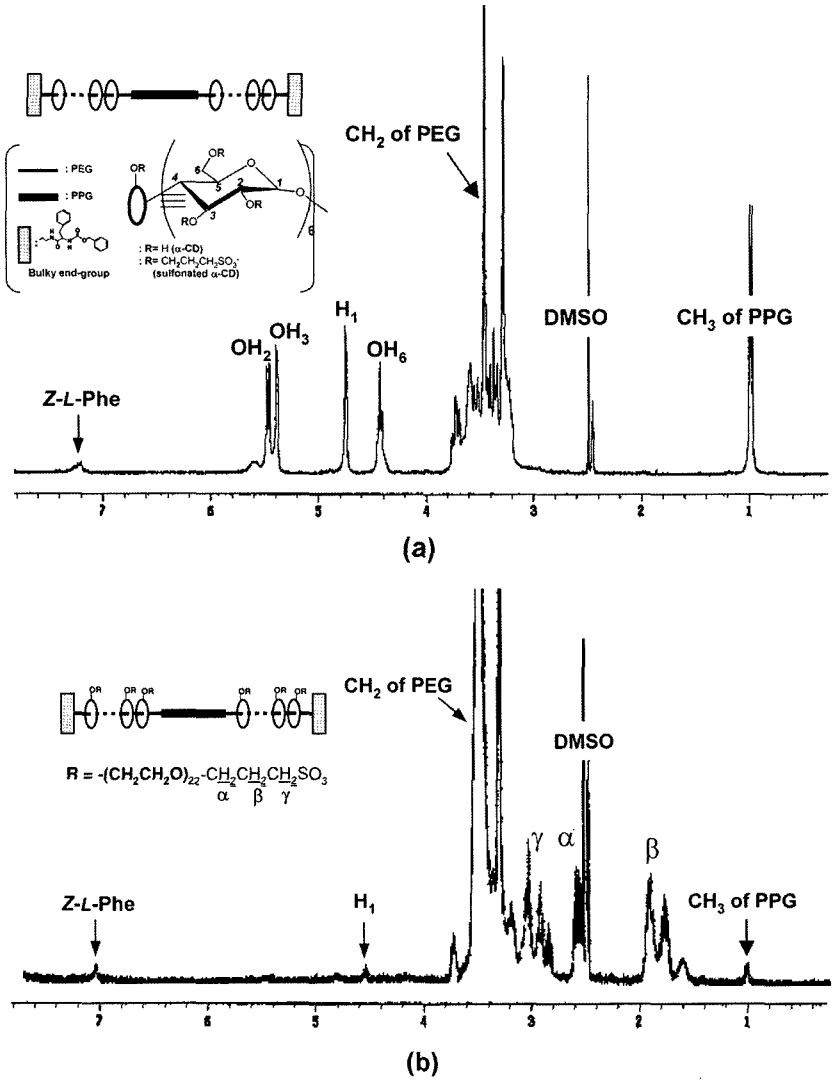

Figure 3. The 300 MHz ^{1}H-NMR spectra of PRx (a) and the PRx-PEG(1.0k)-SO_3 (b).

Characterization of PRx Derivatives-Modified PU. Figure 4 shows that in the nonhydrated state, the incorporated PRx-PEG-SO_3 caused a decrease in contact angle by the 8-35°, compared to the control PU surface. This result indicates that the hydrophilic portion of the PRx-PEG-SO_3 is present on the surface after modification. The hydrated PU/PRx-PEG-SO_3 for 1 d demonstrated a slight increase in contact angles compared with the dried ones, suggesting that the extraction rate of the amphiphilic PRx-PEG-SO_3 from the blended surface presumably was higher than the diffusion rate in the PU matrix. It was reported that the PU surface modified by blending the other amphiphiles showed similar trends.[6] PRx-PEG-SO_3 incorporated surfaces except for the PRx-PEG(0.3k)-SO_3 showed the improved hydrophilicity, compared to the PRx- and the PRx-SO_3-modified surfaces.

In the C_{1s} spectra of ESCA (Figure 5), PU/PRx-SO_3 (P105) showed a slight increase in the ether carbon peak (-C-O-) as compared to the control PU. PU/PRx-PEG(1.0k)-SO_3 (P105) showed a significant increase in the ether carbon peak and the ratio of the ether carbon to alkyl carbon, in addition to the largest exposure of sulfur (1.2%) on the surface. These results suggest that the exposure of the hydrophilic portion and presumably the stable anchorage by the unique properties of PEG in the grafted PEG-SO_3 contribute to these phenomena. The incorporated PRx-PEG-SO_3 may lead to the enhanced hydrophilicity by the change in the surface properties of the PU matrix.

The incorporation of the PRx-PEG-SO_3 (P105) into the PU matrix caused the higher water uptake compared to the control PU, as shown in Figure 4. The water absorption gradually increased with the increasing PEG chain length of the PEG-SO_3, probably owing to the unique hydration effect of PEG. When it compared with PU/PRx and PU/PRx-SO_3, PU/PRx-PEG-SO_3 (P105) showed almost same or higher

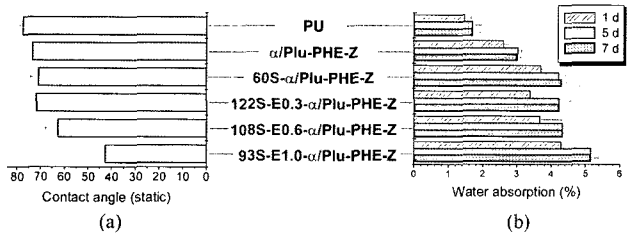

Figure 4. Contact angle and water absorption results of the control and modified PU surfaces. (a) static contact angle data and (b) water adsorption result (mean ± standard error of the mean, n=4).

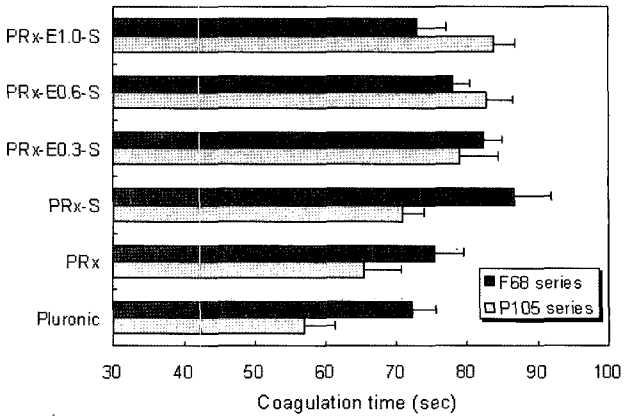

Figure 6. Anticoagulant activity of polyrotaxane derivatives by APTT (mean ± standard error of the mean, n=4).

water absorption, which may be affected by the extraction rate and the diffusion rate of the amphiphiles (PRx or PRx derivatives) in the PU matrix. In addition, the relatively higher extraction was demonstrated in the order of the control (0.3%), PRx (0.6%), PRx-SO_3 (0.7%), and PRx-PEG-SO_3 (1.5-1.7%) in PU substrates. The amount of extraction of PRx-PEG-SO_3 was slightly higher (1.5-1.7%) as the PEG chain lengths increase (0.3 to 1.0k), suggesting that the higher hydrophilicity and exposure on surface of PRx-PEG(1.0k)-SO_3 in the PU matrix may contribute to these phenomena. However, it is also noted that a small amount of PRx-PEGs-SO_3 (1.5-1.7%) was eluted after exposure to water. Thus, the incorporated PRx-PEG-SO_3 showed stable entrapment in the PU matrix with little extraction into the water phase.

Anticoagulant Activities of PRx Derivatives. Figure 6 shows that the anticoagulant activities of PRx derivatives (Pluronic, P105 and F68 series). In P105 series, the anticoagulant activities increased in the order of Pluronic, PRx (PRx-Na), PRx-SO_3, and PRx-PEG-SO_3. Also, the coagulation times of PRx-PEG-SO_3 polymers prolonged gradually with increasing the PEG chain length. This result suggests that both the supramolecular-structured polyrotaxane and the sulfonated groups contribute to the enhancement of anticoagulant activity, and that furthermore, the higher mobility induced by the PEG-SO_3 segments sterically inhibit the intrinsic coagulation cascade. However, the PRx-PEG-SO_3 in F68 model showed lower anticoagulant activity than the PRx-SO_3. It is estimated that the effects of the higher mobility and the heparin-like sulfonated groups postulated by the PEG-SO_3 on the anticoagulant activity are probably inhibited by the stronger intermolecular association between the hydrophobic PPG chains in PRx-PEG-SO_3 with the increase of molecular weight by the introduction of PEG-SO_3. Pluronics of higher molecular weight for given PPG/PEG ratio are known to form micelles more readily.[24] It is also noted that Pluronic, PRx, and PRx-SO_3 in F68 showed the relatively higher activities rather than those in P105, suggesting that higher HLB (Hydrophilic-Lipophilic balance) of F68[25] and lower portion of PEG segments included by α-CDs in PRx (F68) as mentioned at Table I, may contribute to enhance their activities.

Generally, the extent of activity in the various coagulation assays including APTT is influenced by the various physicochemical parameters such as molecular weight, chain length, charge density, and chain mobility.[26-28] These values are conditional on each other and on the respective basic structure, suggesting that the incorporation of PEG-SO_3 to the supramolecular-structured PRx are meaningful for the inhibition of intrinsic coagulation factors. PRx derivatives

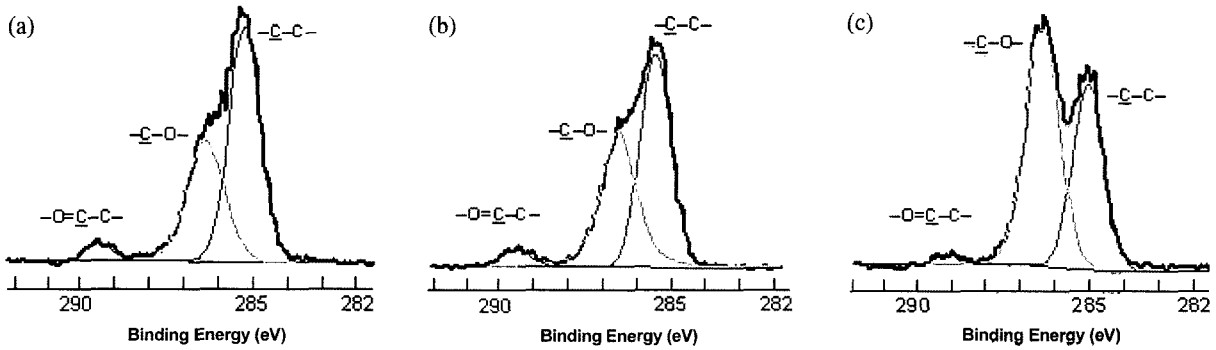

Figure 5. ESCA C_{1s} spectra of the control and modified PU surfaces. (a) PU control, (b) PU/PRx-SO_3 (P105), and (c) PU/PRx-PEG 1.0k-SO_3 (P105).

with sulfonated groups may be applicable to the surface modification of clinically used polymers as one of new materials for blending or coating.

Albumin and Fibrinogen Adsorption. Figure 7 shows the relative amounts of the absorbed albumin and fibrinogen on PU surfaces. Considering that the theoretical amounts of albumin and fibrinogen in a monolayer state are to be about 1 and 2 $\mu g\ cm^{-2}$, respectively, the proteins adsorbed on the all surfaces is estimated to form monolayers because the highest amounts of the absorbed albumin and fibrinogen on the all surfaces were 0.8 and 1.4 $\mu g\ cm^{-2}$.

The amount of adsorbed albumin on the all modified PUs was much higher or same compared with the control PU. Relatively higher hydrophobic surface (PU/PRx, as shown in Figure 5(a)) among the PRx- and PRx derivatives-containing PUs showed an increase in albumin adsorption. It was reported that albumin has a hydrophobic pocket and can conjugate with long hydrophobic alkyl chains.[29] The microdomain surface structure, which is composed of hydrophilic and hydrophobic components, induces the selective adsorption of proteins.[30] The incorporated PEG-SO_3 to PRx (P105 and F68) decreased the albumin adsorption on surface. All the surfaces of F68 series shows a less fibrinogen adsorption compared to the control PU, while those of P105 series demonstrated the higher fibrinogen adsorption. This result suggests that the higher PEG of F68 and the grafted PEG-SO_3 contribute to lower the amount of fibrinogen adsorption. It has been reported that the larger PEG blocks of the amphiphile are more effective as protein repellents.[31] This result suggests that the hydrophilic portions including the sulfonated group-containing α-CDs and PEG segments extend onto the surface and sterically prevent the proteins from closely approaching the surface. Directly sulfonated PU (PU-SO_3) surface is reported to exhibit more fibrinogen adsorption than untreated PU as sulfonate content increases.[32,33] Also, the sulfonated PEG-grafted PU (PU-PEG-SO_3) surface has demonstrated higher albumin and lower fibrinogen adsorption than PU control in plasma.[34]

Though it is difficult to compare with each other precisely due to the surface modification method and the presence of plasma which contains an abundance of proteins. The profile of proteins adsorption of PU/PRx-PEG-SO_3 is considered to be similar to that of PU-PEO-SO_3. It is well known that fibrinogen-coated surfaces are thrombogenic while precoating with albumin makes all surfaces less reactive to platelets.[35,36] In this point of view, F68-containing PRx derivatives can be estimated to be more blood compatible rather than P105 ones. It may be also related with a structural arrangement primarily governed by their stable anchorage of the hydrophobic PPG center blocks of Pluronics in PU matrix. These profiles of protein adsorption might be correlated with the compensatory effects between the supramolecular-structured PRx and the mobile CD derivatives.

bFGF Binding Affinity. Figure 8 shows the fibroblast growth factor (bFGF)-binding affinity with PRx derivatives-modified PU surfaces. Unexpectedly, the binding affinity with the sulfonated PRx (PRx-SO_3) substrate was almost the same with the non-sulfonated PRx substrate. However, the bFGF binding affinity with PRx-PEG-SO_3 substrate was significantly higher than the non-sulfonated PRx and the sulfonated PRx (PRx-SO_3). These results suggest that the

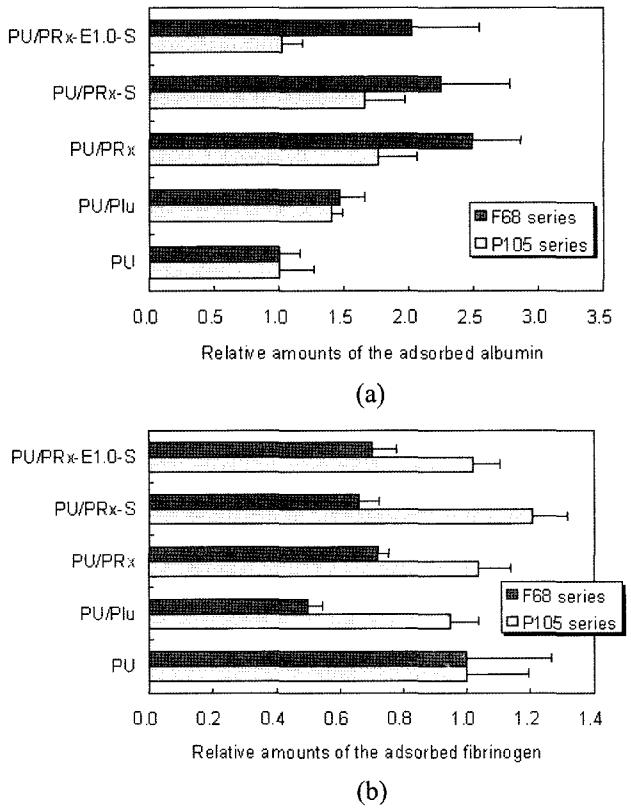

Figure 7. Relative amounts of the absorbed proteins on modified PU surfaces. (a) albumin adsorption behavior and (b) fibrinogen adsorption behavior (mean ± standard error of the mean, n=4).

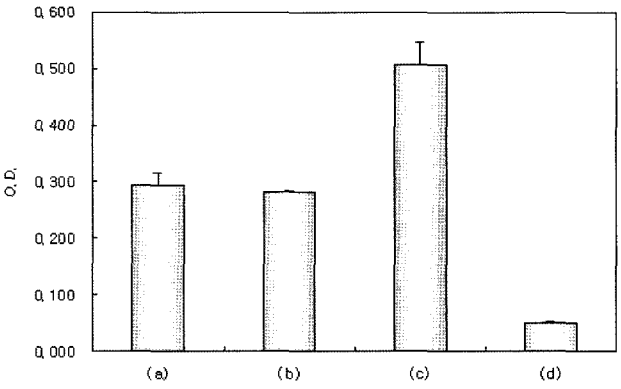

Figure 8. bFGF adhesion of the control and modified PU surface. (a) PU/PRx, (b) PU/PRx-SO_3, (c) PU/PRx-PEG-SO_3, (d) PU control (mean ± standard error of the mean, n=4).

hydrophilicity and the highly dynamic mobility induced by the PEG-SO_3 segments enable PRx-PEG-SO_3 to extend onto the surface and sterically promote the binding affinity with bFGF. Further studies on the ligand-receptor binding and the cell adhesion are now under investigation.

Conclusions

The supramolecular structure of PRx makes it possible to prepare the multivalent PEG-SO_3 grafted polymer (PRx-PEG-SO_3). Sulfonated PEG (PEG-SO_3) grafted polyrotaxanes (PRx-PEG-SO_3) have demonstrated the blood compatibility including the high anticoagulant activity and the low fibrinogen adsorption as well as the high binding affinity with fibroblast growth factor.

These types of polyrotaxane may be applicable not only as one of new surface-modifying biomaterials, but also as a useful matrix of a number of growth factors delivery for cell/tissue engineering applications.

Acknowledgements. This work was supported by a grant from the KOSEF (Korea-Japan International Joint Research Grant, F01-2002-000-20020).

References

(1) R. E. Baier, *Artif. Organs*, **2**, 422 (1978).
(2) R. E. Baier and R. C. Dutton, *J. Biomed. Mater. Res.*, **3**, 191 (1969).
(3) J. L. Brash and T. A. Horbett, *Proteins at Interfaces,* American Chemical Society, Washington DC, 1987.
(4) J. H. Lee, J. Kopecek, and J. D. Andrade, *J. Biomed. Mater. Res.*, **23**, 351 (1989).
(5) K. D. Park, A. Z. Piao, H. Jacobs, T. Okano, and S. W. Kim, *J. Polym. Sci.; Part A: Polym. Chem.*, **29**, 1725 (1991).
(6) J. M. Harris, *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*, Plenum Press, New York, 1992.
(7) T. B. McPherson, A. Kidane, I. Szleifer, and K. Park. *Langmuir*, **14**, 176 (1998).
(8) M. Amiji and K. Park. *Biomaterials*, **13**, 682 (1992).
(9) J. S. Tan, D. E. Butterfield, C. L. Voycheck, K. D. Caldwell, and J. T. Li, *Biomaterials*, **14**, 823 (1993).
(10) T. B. McPherson, H. S. Shim, and K. Park. *J. Biomed. Mater. Res. Appl. Biomater.*, **38**, 289 (1997).
(11) Y. H. Kim, D. K. Han, K. D. Park, and S. H. Kim, *Macromol. Symp.*, **118**, 565 (1997).
(12) K. D. Park, K. Suzuki, W. K. Lee, J. E. Lee, Y. H. Kim, Y. Sakurai, and T. Okano, *ASAIO J.*, **42**, 876 (1996).
(13) K. D. Park, W. K. Lee, J. Y. Yun, D. K. Han, Y. H. Kim, H. M. Kim, and K. T. Kim, *Biomaterials*, **18**, 47 (1997).
(14) J. Folkman, P. B. Weisz, M. M. Joullie, W. W. Li, and W. R. Ewing, *Science*, **243**, 1490 (1989).
(15) M. Fujita, M. Ishihara, M. Simizu, K. Obara, T. Ishizuka, Y. Saito, H. Yura, Y. Morimoto, B. Takase, T, Matsui, M. Kikuchi, T. Maehara, *Biomaterials*, **25**, 699 (2004)
(16) A. Harada, J. Li, and M. Kamachi, *Nature*, **356**, 325 (1992).
(17) A. Harada, M. Okada, J. Li, and M. Kamachi, *Macromolecules*, **28**, 8406 (1995).
(18) A. Harada, J. Li, and M. Kamachi, *Nature*, **364**, 516 (1993).
(19) J. H. Lee, P. A. Martic, and J. S. Tan, *J. Colloid Interf. Sci.*, **131**, 252 (1989).
(20) H. D. Park, W. K. Lee, T. Ooya, K. D. Park, Y. H. Kim, and N. Yui. *J. Biomed. Mater. Res.*, **60**, 186 (2002).
(21) H. D. Park, W. K. Lee, T. Ooya, K. D. Park, Y. H. Kim, and N. Yui. *J. Biomed. Mater. Res.*, **66**, 596 (2003).
(22) L. Thomas, *Partielle Thromboplastinzeit, Labor und Diagnose Med*, Verlagsgesellschaft, Marburg, 1992.
(23) P. K. Smith, R. I. Krohn, and G. T. Hermanson, *et al.*, *Anal. Biochem.*, **150**, 76 (1985).
(24) P. Alexandridis, J. F. Holzwarth, and T. A. Hatton, *Macromolecules*, **27**, 2414 (1994).
(25) P. Alexandridis and T. A. Hatton, *Colloid Surfaces A*, **96**, 1 (1995).
(26) S. Alban, J. Kraus, and G. Franz, *Arzneim -Forsch/Drug Res.*, **42**, 1005 (1992).
(27) S. Alban and G. Franz, *Pure Appl. Chem.*, **66**, 2403 (1994).
(28) T. Nishino, Y. Aizu, and T. Nagumo, *Thromb. Res.*, **64**, 723 (1991).
(29) M. S. Munro, A. J. Quattrone, S. R. Ellsworth, P. Kulkarni, and R. C. Eberhart, *Trans. Amer. Soc. Artif. Intern. Organs*, **27**, 449 (1981).
(30) T. Okano, S. Nishiyama, I. Shinohara, T. Akaike, Y. Sakurai, K. Kataoka, and T. Tsuruta, *J. Biomed. Mater. Res.*, **15**, 393 (1981).
(31) J. T. Li and K. D. Caldwell, *Colloid Surfaces B*, **7**, 9 (1996).
(32) T. G. Grasel and S. L. Cooper, *J. Biomed. Mater. Res.*, **23**, 311 (1989).
(33) J. P. Santerre, N. H. VanderKamp, and J. L. Brash, *J. Biomed. Mater. Res.*, **26**, 39 (1992).
(34) D. K. Han, K. D. Park, G. H. Ryu, U. Y. Kim, B. G. Min, and Y. H. Kim, *J. Biomed. Mater. Res.*, **30**, 23 (1989).
(35) S. W. Kim, R. G. Lee, H. Oster, D. Coleman, and J. D. Andrade, *Trans. Amer. Soc. Artif. Intern. Organs*, **20**, 449 (1974).
(36) E. W. Salzman, J. Lindon, D. Brier, and E. W. Merril, *Ann. NY Acad. Sci.*, **283**, 114 (1997).