DOI: 10.1021/ma902029s

Macromolecules
COMMUNICATION TO THE EDITOR

Nonfouling Polyampholytes from an Ion-Pair Comonomer with Biomimetic Adhesive Groups

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Received September 10, 2009
Revised Manuscript Received November 10, 2009

Nonfouling surfaces play a very important role for the development of biosensors,[1] medical implants,[2] and drug delivery vehicles.[3] However, few materials and modification methods have been reported to improve the nonfouling properties of surfaces effectively and conveniently. Among these reported materials, zwitterionic polymers, with both positive and negative charges on the side chains, showed excellent nonfouling properties in our previous studies.[4−7] On the basis of these studies, it was hypothesized that a nanometer-scale homogeneous mixture of balanced charge groups will present protein-resistant properties. Recently, this assumption has been proved on hydrogels via free radical polymerization,[8] polymer thin films[9] via surface-initiated atom transfer radical polymerization (SI-ATRP), and polymer thin films via self-assembly.[10,11] The ratios of positively to negatively charged monomers need to be optimized to achieve 1:1 on surfaces for most of these cases.

Polyampholytes are the synthetic analogues of naturally occurring biological molecules such as proteins and have been used in areas such as lithographic film,[12] emulsion formulation,[13] and drag reduction.[14] Extensive studies have been done on the control of the cationic−anionic ratio on the polymer side chains. Salamone et al.[15−17] reported the synthesis of polyampholytes with equimolar polyampholyte derived from cationic−anionic monomer pairs. Peiffer et al.[18] reported the synthesis of polyampholytes without self-neutralized charge from equimolar charged monomers in the presence of nonpolymerizable counterions. McCormick et al.[19] showed a high alternating tendency of the charged monomers in the copolymerization of sodium 2-acrylamido-2-methylpropanesulfonate and (2-acrylamido-2-methylpropyl)-dimethylammonium chloride. Yang et al.[20] also reported that 1:1 copolymer was obtained via the ion-pair method.

When coming to the methods of surface modification, both "graft from" and "graft to" methods have been used. The former gives higher packing densities and well-controlled thicknesses,[5,21−24] whereas the latter is more convenient for practical applications.[25−28] 3,4-Dihydroxyphenyl-L-alanine (DOPA) and its derivatives inspired from the adhesive proteins found in mussel have been successfully incorporated into various synthetic polymers as the "graft to" anchor groups.[25] Our previous studies demonstrated that polybetain incorporated with a catechol group can be successfully grafted to a surface with nonfouling properties.[26]

In this work, two polyampholytes of equimolar charged monomers with two types of catecholic anchor groups were synthesized via ATRP and free radical polymerization of the ion-pair comonomer. Two resulting polyampholytes are nonfouling without the need to optimize their surface ratios as in the case of randomly mixed charge nonfouling materials. The molecular weight and polydispersity index (PDI) of the polyampholytes were determined by using an aqueous gel permeation chromatography (GPC). The film thickness of the polymers attached on surfaces was measured by an ellipsometer. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) was used to monitor functional groups on the surface qualitatively. Electron spectroscopy for chemical analysis (ESCA) was employed to determine the surface composition quantitatively. The protein-resistant properties were determined by measuring protein adsorption from fibrinogen (Fg), lysozyme (Lyz), and bovine serum albumin (BSA) using a surface plasmon resonance (SPR) sensor.

The ion-pair comonomer (METMA·MES) was first synthesized from [2-(methacryloyloxy)ethyl]trimethylammonium chloride and 2-sulfoethyl methacrylate using a similar method reported before,[15] which is described in the Supporting Information. Previous studies showed that polyampholytes prepared in solution without any nonpolymerizable ions (such as inorganic cations and anions) have a tendency to be alternating as a result of strong electrostatic attractive forces acting between two opposite charged monomers.[29] Therefore, after polymerization of the ion-pair monomers, equal amounts of the monomers can be incorporated in these high charge density copolymers.

Two initiators (initiator **1** and initiator **2**) with protected catecholic anchor groups were designed and synthesized as described in the Supporting Information (Scheme S1 and experimental details). The chemical structures of initiator **1** and initiator **2** are shown in Figure 1. It can be seen that initiator **1** can be used to initiate atom transfer radical polymerization (ATRP) while initiator **2** is a typical initiator for free radical polymerization. Both of them are incorporated with adhesive catechol groups for an anchor. It should be mentioned that the protection of catecholic oxygens by the *tert*-butyldimethylsilyl (TBDMS) groups can avoid side reactions during the polymerization and keep the adhesive polyampholytes stable before using.[30]

Therefore, polymer **I** (M_n 19 143) was obtained by ATRP of METMA·MES from initiator **1**, while polymer **II** (M_n 28 276) came from the free radical polymerization of METMA·MES from initiator **2**. The detailed conditions of polymerization and the characterization of the polymers by gel permeation chromatography (GPC) are described in the Supporting Information. Because of the different structures of initiator **1** and initiator **2**, the adhesive groups will be located at different positions on the polymer chains. For polymer **I**, the two catechol groups are in the middle of the chain. For polymer **II**, the catecholic adhesive groups are located at the end(s) of the polymer chain. For free radical polymerization, two common types of termination reactions are combination and disproportionation. Since the free radicals at the both ends of the growing poly(METMA·MES) chain are sterically hindered due to the presence of methyl groups, termination reaction by combination is impeded (catechol groups are at both ends), and termination reaction by disproportionation predominates (cathechol groups are only at one end). Therefore, the main product from this work may be the polymer with one catechol group at one end of the chain. Both of these polyampholytes were deprotected by tetrabutylammonium fluoride (TBAF), a mild deprotecting reagent, to remove the TBDMS

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 Published on Web 12/03/2009

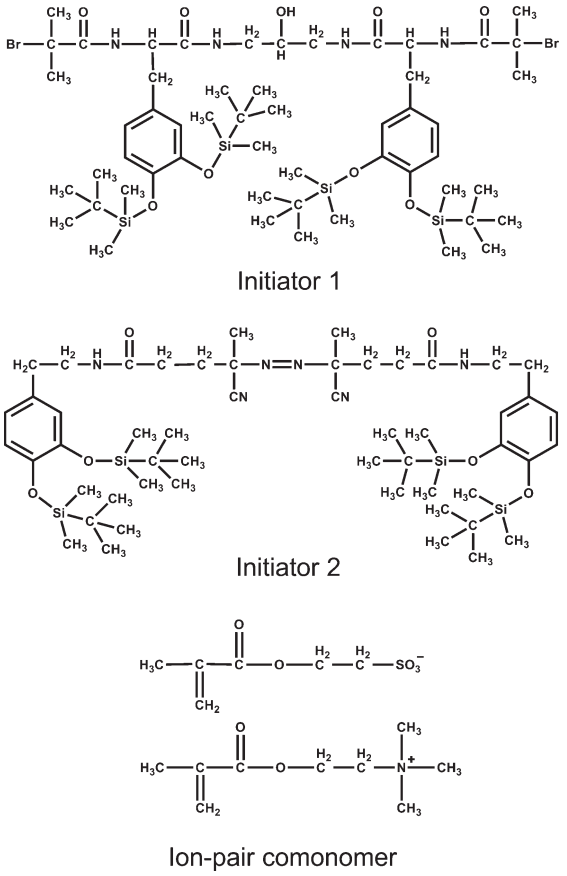

Figure 1. Chemical structures of initiator **1**, initiator **2**, and the ion-pair comonomer.

Table 1. Surface Characterizations (Average ± SD)

surfaces modified by	polymer **I**	polymer **II**
N/S ratio by ESCA (n = 4)	1.00 ± 0.05	0.96 ± 0.06
film thickness by ellipsometer (nm) (n = 10)	5.75 ± 2.20	6.94 ± 1.88

groups before their usage for surface modification. A THF−water system was employed when the adhesive polymers were anchored to gold used as a model surface which is described further in the Supporting Information.

The film thickness of the modified surfaces was measured by an ellipsometer. Results are summarized in Table 1. Messersmith et al.[28] reported a 3−4 nm film of DOPA−PEG polymer adhered onto a titanium oxide surface. Herein more than 5 nm film of the polymers grafted onto gold gives a good evidence that both polyampholytes adhere well on the surfaces. In addition, the thicker film of polymer **II** than that of polymer **I** can be explained by the fact that the molecular weight of polymer **II** is higher than that of polymer **I**. The modified surfaces were also characterized by ATR-FTIR. Figure S1 shows the typical ATR-FTIR spectra of the surfaces modified by polymer **I** and polymer **II**. The strong absorbent peaks at 1039, 1180, and 1729 cm^{-1} correspond to SO_3, C−O, and C=O stretches, which is consistent with our previous studies.[22] Furthermore, ESCA was employed to determine their surface composition quantitatively. The ratio of the atomic percentage of nitrogen and sulfur was used to quantify the ratio of METMA and MES on the polymer chains. These ratios are summarized in Table 1. It can be seen from Table 1 that the calculated N/S ratios of polymer **I** and polymer **II** on the surfaces are 1.00 and 0.96, respectively. Then, it can be concluded that the statistical METMA/MES ratios of polymer **I** and polymer **II** are both 1:1, which is consistent with the studies of Salamone et al.[15] Therefore, two homogeneous mixed polyampholytes with exact

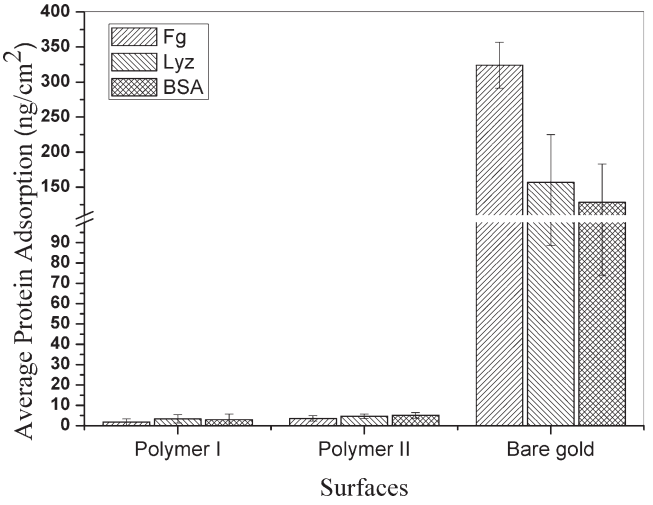

Figure 2. Adsorption of Fg, Lyz, and BSA to the surfaces modified by polymer **I** and polymer **II** in comparison to a bare gold surface. Error bar represents the standard derivations of the mean ($n \geq 4$).

overall charge neutrality have been obtained. Representative ESCA spectra can be found in the Supporting Information (Figures S2 and S3).

The protein-resistant properties of the modified surfaces were tested by a SPR sensor,[31] which is ideal for measuring quantitative protein adsorption on a surface. Fibrinogen (Fg), lysozyme (Lyz), and bovine serum albumin (BSA) were selected as test proteins. Fg is a soft and negatively charged protein, which can easily adsorb onto a wide range of materials. Lyz is a hard and positively charged protein while albumin is natural abundant in the body. The adsorption of Fg, Lyz, and BSA was measured simultaneously in a four-channel SPR. Typical SPR sensorgrams can be found in the Supporting Information (Figures S4−S6). The summarized results are shown in Figure 2. The measured amounts of adsorbed Fg, Lyz, and BSA are 1.7 ± 1.6, 3.3 ± 2.0, and 2.9 ± 2.8 ng/cm^2 for polymer **I**, and 3.5 ± 1.3, 4.6 ± 1.1, and 5.0 ± 1.4 ng/cm^2 for polymer **II**, respectively. Thus, both surfaces modified by polymer **I** and polymer **II** show very good nonfouling properties. The nonfouling behaviors of the coated surfaces can be explained by the strong hydration layer on the surface coming from the neutral charged and the nearly perfect alternating METMA and MES on the side chains of the polymers.[32] In addition, it should be mentioned that polymer **II** modified surfaces gave slightly higher nonspecific protein adsorption than that of polymer **I**. That can be attributed to the polymer structures discussed before. The main composition of polymer **II** results from termination reaction by disproportionation, which has only one catechol group at the end of the polymer chain. In comparison to polymer **II**, polymer **I** has two catechol groups for stronger binding[28] and two nonfouling chains for higher chain packing density, leading to denser adlayers and lower protein adsorption. Chen et al.[33] reported the doubled density of the two-chain grafting from the surface as compared to the one-chain grafting. In addition, for a small amount of polymer **II** with two catechol groups resulting from termination by combination, their binding onto an Au surface is not expected to be strong to hold both anchors at the far ends of a polymer chain. If only one end is attached, then unbounded catecholic groups at the other end will lead to some nonspecific protein adsorption.

In summary, two adhesive polyampholytes were synthesized by the polymerization of an ion-pair comonomer using two types of catecholic initiators. ESCA results show the N/S ratios of 1 and 0.96 for the gold surfaces modified by polymer **I** and polymer **II**, respectively. These neutral charged surfaces give excellent nonfouling properties from protein solutions of Fg, Lyz, and

BSA. This strategy to prepare ion-pair nonfouling polymers is convenient for surface attachment without adjusting the ratio of charged monomers.

Acknowledgment. This work is supported by the Office of Naval Research (N000140711036). Guozhu Li acknowledges a fellowship from the China Scholarship Council. The XPS experiments were performed at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO) supported by NIBIB Grant EB02027.

Supporting Information Available: Experimental details, typical ATR-FTIR spectra and ESCA survey scans of the modified surfaces, and typical SPR sensorgrams of protein adsorption on these surfaces are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252* (5009), 1164–1167.
(2) Ratner, B. D.; Bryant, S. J. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 41–75.
(3) Langer, R. *Science* **2001**, *293* (5527), 58–59.
(4) Chen, S.; Zheng, J.; Li, L.; Jiang, S. *J. Am. Chem. Soc.* **2005**, *127*, 14473–14478.
(5) Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. *Biomacromolecules* **2008**, *9* (5), 1357–1361.
(6) Zhang, Z.; Chao, T.; Chen, S.; Jiang, S. *Langmuir* **2006**, *22*, 10072–10077.
(7) Zhang, Z.; Chen, S.; Jiang, S. *Biomacromolecules* **2006**, *7* (12), 3311–3315.
(8) Chen, S. F.; Jiang, S. Y. *Adv. Mater.* **2008**, *20* (2), 335–338.
(9) Bernards, M. T.; Cheng, G.; Zhang, Z.; Chen, S.; Jiang, S. *Macromolecules* **2008**, *41* (12), 4216–4219.
(10) Chen, S. F.; Yu, F. C.; Yu, Q. M.; He, Y.; Jiang, S. Y. *Langmuir* **2006**, *22* (19), 8186–8191.
(11) Chen, S.; Cao, Z.; Jiang, S. *Biomaterials* **2009**, *30* (29), 5892–5896.
(12) Foss, R. P. Acrylic amphoteric polymers. U.S. Patent 4749762, **1988**.
(13) Farwaha, R.; Currie, W. Amphoteric surfactants and copolymerizable amphorteric surfactants for use in latex paint. U.S. Patent 5240982, **1993**.
(14) Mumick, P. S.; Welch, P. M.; Salazar, L. C.; McCormick, C. L. *Macromolecules* **1994**, *27* (2), 323–331.
(15) Salamone, J. C.; Quach, L.; Watterson, A. C.; Krauser, S.; Mahmud, M. U. *J. Macromol. Sci., Chem.* **1985**, *A22* (5−7), 653–664.
(16) Salamone, J. C.; Mahmud, N. A.; Mahmud, M. U.; Nagabhushanam, T.; Watterson, A. C. *Polymer* **1982**, *23* (6), 843–848.
(17) Salamone, J. C.; Watterson, A. C.; Hsu, T. D.; Tsai, C. C.; Mahmud, M. U. *J. Polym. Sci., Part C: Polym. Lett.* **1977**, *15* (8), 487–491.
(18) Peiffer, D. G.; Lundberg, R. D. *Polymer* **1985**, *26* (7), 1058–1068.
(19) McCormick, C. L.; Johnson, C. B. *Macromolecules* **1988**, *21* (3), 686–693.
(20) Yang, J. H.; Jhon, M. S. *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33* (15), 2613–2621.
(21) Ma, H. W.; Hyun, J. H.; Stiller, P.; Chilkoti, A. *Adv. Mater.* **2004**, *16* (4), 338–341.
(22) Li, G. Z.; Xue, H.; Cheng, G.; Chen, S. F.; Zhang, F. B.; Jiang, S. Y. *J. Phys. Chem. B* **2008**, *112* (48), 15269–15274.
(23) Yang, W.; Chen, S.; Cheng, G.; Vaisocherová, H.; Xue, H.; Keefe, A.; Li, W.; Zhang, J.; Jiang, S. *Langmuir* **2008**, accepted.
(24) Azzaroni, O.; Brown, A. A.; Huck, W. T. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1770–1774.
(25) Dalsin, J. L.; Hu, B.-H.; Lee, B. P.; Messersmith, P. B. *J. Am. Chem. Soc.* **2003**, *125*, 4253–4258.
(26) Li, G. Z.; Cheng, G.; Xue, H.; Chen, S. F.; Zhang, F. B.; Jiang, S. Y. *Biomaterials* **2008**, *29* (35), 4592–4597.
(27) Statz, A. R.; Meagher, R. J.; Barron, A. E.; Messersmith, P. B. *J. Am. Chem. Soc.* **2005**, *127*, 7972–7973.
(28) Dalsin, J. L.; Lin, L. J.; Tosatti, S.; Voros, J.; Textor, M.; Messersmith, P. B. *Langmuir* **2005**, *21* (2), 640–646.
(29) Kudaibergenov, S. E. *Polyampholytes: Synthesis, Characterization, and Application*; Springer: Berlin, 2002.
(30) Sever, M. J.; Wilker, J. J. *Tetrahedron* **2001**, *57* (29), 6139–6146.
(31) Homola, J. *Chem. Rev.* **2008**, *108* (2), 462–493.
(32) He, Y.; Hower, J.; Chen, S. F.; Bernards, M. T.; Chang, Y.; Jiang, S. Y. *Langmuir* **2008**, *24* (18), 10358–10364.
(33) Chen, R. X.; Feng, C.; Zhu, S. P.; Botton, G.; Ong, B.; Wu, Y. L. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44* (3), 1252–1262.