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Precise control of surface electrostatic forces on polymer brush layers with opposite charges for resistance to protein adsorption

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ABSTRACT

Various molecular interaction forces are generated during protein adsorption process on material surfaces. Thus, it is necessary to control them to suppress protein adsorption and the subsequent cell and tissue responses. A series of binary copolymer brush layers were prepared via surface-initiated atom transfer radical polymerization, by mixing the cationic monomer unit and anionic monomer unit randomly in various ratios. Surface characterization revealed that the constructed copolymer brush layers exhibited an uniform super-hydrophilic nature and different surface potentials. The strength of the electrostatic interaction forces operating on these mixed-charge copolymer brush surfaces was evaluated quantitatively using force-versus-distance (*f-d*) curve measurements by atomic force microscopy (AFM) and probes modified by negatively charged carboxyl groups or positively charged amino groups. The electrostatic interaction forces were determined based on the charge ratios of the copolymer brush layers. Notably, the surface containing equivalent cationic/anionic monomer units hardly interacted with both the charged groups. Furthermore, the protein adsorption force and the protein adsorption mass on these surfaces were examined by AFM *f-d* curve measurement and surface plasmon resonance measurement, respectively. To clarify the influence of the electrostatic interaction on the protein adsorption behavior on the surface, three kinds of proteins having negative, positive, and relatively neutral net charges under physiological conditions were used in this study. We quantitatively demonstrated that the amount of adsorbed proteins on the surfaces would have a strong correlation with the strength of surface-protein interaction forces, and that the strength of surface-protein interaction forces would be determined from the combination between the properties of the electrostatic interaction forces on the surfaces and the charge properties of the proteins. Especially, the copolymer brush surface composed of equivalent cationic/anionic monomer units exhibited no significant interaction forces, and dramatically suppressed the adsorption of proteins regardless of their charge properties. We conclude that the established methodology could elucidate relationship between the protein adsorption behavior and molecular interaction, especially the electrostatic interaction forces, and demonstrated that the suppression of the electrostatic interactions with the ionic functional groups would be important for the development of new polymeric biomaterials with a high repellency of protein adsorption.

1. Introduction

Advanced control of protein adsorption on the material surface, including effective suppression, is one of the most fundamental elements in several fields, such as biomaterials, bio-sensing, pharmaceuticals, food productions. In the biomaterials field in particular, protein adsorption onto the material surface is vitally important because it is an initial reaction induced immediately by contact with artificial foreign materials and biological environments, and the properties of adsorbed protein layers are significant factors that determine the following biological responses [1–3]. Protein adsorption proceeds through several steps, and various molecular interaction forces, such as surface-protein interactions and protein-protein interactions, are closely related to the formation of the adsorbed protein layer [4,5]. The nature of interaction forces operating during this process determines the final characteristics of the adsorbed protein layer on the material surface.

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Hence, it is important to describe protein adsorption in terms of the actual molecular interactions operating on the surface or between molecules. In our previous studies, we established a novel analytical methodology to understand protein adsorption from the perspective of molecular interaction forces, by effectively integrating systematically fabricated polymer brush surfaces with atomic force microscopy (AFM), the nano-force analysis technique [6]. Polymer brush layers enable wide control of the surface properties by the chemical structure of monomer units [7–11], and AFM allows the quantitative evaluation of various interaction forces by using probes modified by appropriate molecules [12–17]. Using AFM, the contribution of electrostatic and hydrophobic interactions can be investigated separately using the zwitterionic, cationic, anionic, and hydrophobic polymer brush surface systems, and such interactions play a significant role in inhibiting the spontaneous detachment of proteins from surfaces, leading to high protein adsorption. Furthermore, it has been suggested that the zwitterionic polymer structure on biomaterial surfaces is essential for eliminating protein adsorption because such surfaces have no significant molecular interaction forces. Here, this new analytical methodology has further potential for elucidating the relationship between protein adsorption behavior and molecular interaction forces. As one of the ideas that take advantage of the polymer brush layer that enables the regulation of its surface structure and properties, it is considered that the magnitude of the molecular interaction forces generated around the surface can be controlled by preparing binary copolymer brush layers using two appropriate monomer units and modulating their composition. For example, using AFM analysis, it has been shown that the cationic poly(2-trimethylammoniumethyl methacrylate (TMAEMA)) and anionic poly(3-sulfopropyl methacrylate (SPMA)) brush layers generate only electrostatic interactions near the surface [6]. Thus, it has been hypothesized that the strength of electrostatic interactions can be controlled by using a series of binary copolymer brush surfaces composed of TMAEMA and SPMA units. In fact, several groups have attempted to modify surface charge using either binary copolymer brush layers composed of oppositely charged monomer units in different ratios, or layer-by-layer assemblies composed of oppositely charged polyelectrolytes. Notably, pseudo-zwitterionic copolymer brush surfaces composed of equivalent oppositely charged monomer units or layer-by-layer assemblies with zero net charge exhibit non-fouling (protein-resistant) properties [18–20] as well as the polymeric surfaces containing zwitterionic monomer units, such as phosphorylcholine-, sulfobetaine-, and carboxybetaine-based monomers [21–23]. In the present study, we prepared binary copolymer brush layers via surface-initiated atom transfer radical polymerization (SI-ATRP) by mixing cationic TMAEMA and anionic SPMA units randomly in various ratios, to investigate the role of electrostatic interaction forces in protein adsorption over a wide range. These copolymer brush layer systems enable control over the proportion of positive and negative charges, without changing the basic surface structure and hydrophilic nature. We quantitatively evaluated the strength of electrostatic interaction forces on these mixed-charge copolymer brush surfaces by force-versus-distance (*f*-*d*) curve measurements from AFM, using probes modified with charged functional groups. Furthermore, the direct interaction forces with proteins (i.e., the protein adsorption forces) and the protein adsorption mass were quantitatively analyzed for three proteins with different net charges. This study systematically clarifies the quantitative relationship between the strength of electrostatic interaction forces generated on the surfaces and protein adsorption, and provides evidence for the effectiveness of balanced charge structure on the protein repellent property, in terms of molecular interaction forces.

2. Materials and methods

2.1. Materials

TMAEMA and SPMA were purchased from Tokyo Chemical Industry (Tokyo, Japan). Copper(I) bromide (CuBr), 2,2'-bipyridyl (Bpy), and potassium chloride (KCl) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ethyl-2-bromoisobutyrate (EBIB), albumin from bovine serum, lysozyme from chicken egg white, and γ-globulin from bovine blood were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other organic reagents and solvents were commercially available in extra-pure grade and used without further purification. Silicon wafers were purchased from Furuuchi Chemical (Tokyo, Japan); their surfaces were coated with ~10-nm-thick SiO_2 layers.

2.2. Preparation of binary copolymer brush surfaces

Poly(TMAEMA-*co*-SPMA) (PTS) brush layers were prepared on the initiator-immobilized substrates by SI-ATRP using TMAEMA and SPMA, as described previously [24]. Briefly, a surface-immobilizing ATRP initiator, (10-(2-bromo-2-methyl)propionyloxy)decyltrichlorosilane (BrC10TCS), was synthesized and immobilized on the silicon substrates, as described previously [25]. Predetermined amounts of CuBr, Bpy, KCl, and the monomers were dissolved in degassed methanol/water mixture (50/50, v/v), at a final monomer concentration of 0.50 mol/L. KCl was added to the solution at the same concentration as that of the monomers (0.50 mol/L) in order to prevent repulsive or attractive interactions among the ionic units during polymerization in the aqueous medium. Then, the BrC10TCS-immobilized substrates and EBIB, as the free initiator, were simultaneously placed into the solution to initiate SI-ATRP. Polymerization was performed with stirring at 20 °C for 24 h. Then, the substrates were removed from the solution, successively rinsed with water, and dried under nitrogen stream. The target degree of polymerization was set at 50 based on the [monomer]/[free initiator] ratio in the feed. The monomer compositions ([TMAEMA]/[SPMA]) were set at 100/0, 75/25, 50/50, 25/75, and 0/100 in the feed; the ratios were indicated after the abbreviation of polymer (i.e., PTS-100/0, PTS-75/25, PTS-50/50, PTS-25/75, and PTS-0/100), when necessary. The conversion of monomers to polymers was determined by proton nuclear magnetic resonance (^{1}H NMR) (α-400; JEOL, Tokyo, Japan) in deuterium oxide. The chemical structure of the PTS brush layer is shown in Fig. 1.

2.3. Surface characterization

The elemental compositions of the PTS brush surfaces were determined by X-ray photoelectron spectroscopy (XPS) (AXIS-Hsi;

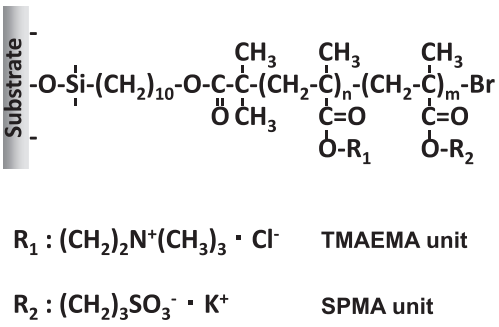

Fig. 1. Chemical structure of PTS brush layer.