Interaction Forces Related to Protein Adsorption on Polymer Brush Surfaces

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Statement of Purpose: Protein adsorption on the biomaterials surface is an important factor that determines subsequent biological responses, including cellular reactions. In particular, unfavarable protein adsorption on the biomaterials surface may cause several serious problems. Therefore the protein adsorption behavior should be precisely understood for the development of biomaterials. Various intermolecular and surface forces between the materials surface and protein are generated. This study aimed to understand protein adsorption behavior in terms of interaction force suchc as hydrophobic interaction, electrostatic interaction, hydrogen bond. Well-defined structure of polymer brush surfaces with systematic physicochemical surface properties were prepared by surface-initiated atom transfer radical polymerization (SI-ATRP) technique. Atomic force microscopic (AFM) technique was applied to directly measure the interaction force operating between two surfaces in aqueous medium down to the few nanonewtons (nN) range. Correlation between interaction force and protein adsorption at the polymer brush surface is discussed.

Methods: Five kinds of monomers; 2-methacryloyloxyethyl phosphorylcholine (MPC, zwitterionic), trimethylammoniumethyl methacrylate (TMAEMA, cationic), 3-sulfoprpyl methacrylate (SPMA, anionic), 2-hydroxyethyl methacrylate (HEMA, hydrophilic), *n*-butyl methacrylate (BMA, hydrophobic) were polymerized from initiator-immobilized substrate or silica particles (diameter: 20 μm) by SI-ATRP method [1]. Surface structure and properties of the polymer brush surfaces were analyzed by X-ray photoelectron spectroscopy (XPS), AFM, spectroscopic ellipsometry, water contact angle measurement, and ζ-potential measurement. The adsorbed amount of albumin (pI 4.8) from bovine serum and lysozyme (pI 11.1) from chicken egg white on polymer brush surfaces was quantified by surface plasmon resonance (SPR) measurement. Polymer-grafted silica particle was glued at the end of a tip-less AFM cantilever [2]. The interaction force between two polymer brush surfaces with the same monomer unit was evaluated by force-versus-distance curve in the AFM measurement mode. Force curve measurement was performed in phosphate-buffered saline (PBS; pH 7.4, I = 0.15 M), PBS diluted by 100, and pure water.

Results: The peaks attributed to each monomer unit were observed in the XPS chart of polymer brush surfaces. AFM height image showed that the surface roughness turned out to be low. The graft density of polymer brush layers calculated from the ellipsometric thickness were high enough to form highly dense polymer brush layers. From the receding contact angle in the dynamic contact angle measurement, the surface free energy in wet state turned out to be high, except for poly(BMA) brush surface. From the ζ-potential measurement, the surface potential turned out to be different by the monomer units. From these results, highly dense polymer brush surfaces with systematic pysicochemical properties were prepared. Figure 1 shows the relationship between the amount of adsorbed proteins and the thickenss of polymer brush layers. On the zwitterionic and hydrophilic polymer brush surfaces, both proteins hardly adsorbed. In the case of the hydrophobic polymer brush surfaces, amount of adsorbed proteins was almost constant with increasing thickness. On the cationic polymer brush surfaces, large amount of albumin (negatively charged protein at pH 7.4) adsorbed, whereas lysozyme (positively charged protein at pH 7.4) hardly adsorbed. The anionic polymer brush surface showed opposite results to the cationic polymer brush surface. Figure 2 shows the force-versus-distance curves of cationic and anionic polymer brush surfaces. In pure water, long-range repulsion was observed, whereas the repulsion weakened with increase of salt concentration at both surfaces. Therefore, these repulsion would originated from electrostatic forces, and it is considered that in the case of these surfaces, protein adsorption are induced by electrostatic interaction between protein and surface.

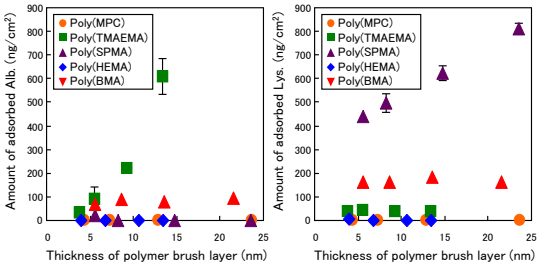

Figure 1. Amount of adsorbed proteins on the polymer brush surfaces.

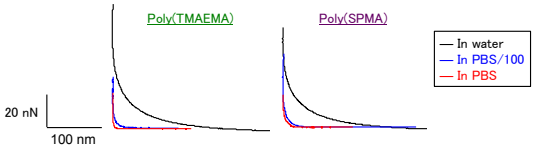

Figure 2. Force-versus-distance curves recorded on the approach for symmetric systems of the cationic and anionic polymer brush surfaces.

Conclusions: Amount of adsorbed proteins depended on physicochemical property of surfaces or charge of proteins. Electrostatic interaction operated on the cationic and anionic surfaces, and it is considered that electrostatic interaction induced protein adsorption on these surfaces even under the physiological conditions. Analysis of interaction force operating on protein repellent surfaces would lead to proposal of new design concept for biomaterials development.

References: [1] Y. Inoue. Colloids Surf. B: Biointerfaces 2010;81:350-357 [2] R. Raiteri. Colloids Surf. A: Physicochem Eng Asp 1998;136:191-197