Surface Interaction Forces Governing Protein Adsorption Analyzed by Direct Force Measurement

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Statement of Purpose: Protein adsorption on the biomaterials surface is one of the initial and important events that determine subsequent biological responses, including cellular reactions. Therefore the protein adsorption behavior should be precisely understood for the development of regenerative medicine or novel biomedical devices. Various intermolecular and surface forces generating between the materials surface and proteins are governing the protein adsorption behavior. The objective of this study is to clarify protein adsorption mechanism on the basis of the interaction forces such as hydrophobic interaction and electrostatic interaction. Atomic force microscopy (AFM) technique was applied to directly measure the nano-forces operating between two surfaces. In order to analyze the protein adsorption behavior in terms of the interaction forces operating on a materials surface, well-characterized surfaces with the precise structure and controlled properties must be prepared as model surfaces. In this regard, polymer brush surfaces were prepared by surface-initiated atom transfer radical polymerization (SI-ATRP) using systematically selected monomers. The relationship between the protein adsorption and interaction force was investigated.

Methods: Polymer brush layers were prepared on the initiator-immobilized substrates or silica particles (diameter: 20 μm) by SI-ATRP method using 2-methacryloyloxyethyl phosphorylcholine (MPC, zwitterionic), 2-trimethylammonium ethyl methacrylate (TMAEMA, cationic), 3-sulfopropyl methacrylate (SPMA, anionic), and *n*-butyl methacrylate (BMA, hydrophobic). Surface structure and properties were analyzed by 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 was quantified by surface plasmon resonance measurement. A silica particle with the polymer brush layers was immobilized at the end of a probeless AFM cantilever, and the interaction force operating between the same kind of polymer brush surfaces was evaluated by the force-versus-distance (*f*-*d*) curve measurement using this probe.

Results: It was confirmed that the highly dense polymer brush surfaces with controlled physicochemical properties were prepared from the results of surface characterization. The amount of adsorbed proteins was plotted against the ellipsometric thickness of polymer brush layers (Fig. 1). On the poly(MPC) brush surfaces, both proteins hardly adsorbed. In the case of the poly(BMA) brush surfaces, the amount of adsorbed proteins was almost constant with increasing thickness. On the poly(TMAEMA) brush surfaces, a large amount of albumin adsorbed, whereas lysozyme hardly adsorbed. The poly(SPMA) brush surface showed opposite results to the cationic polymer brush surface. Fig. 2 shows the *f*-*d* curves recorded for the symmetric system of polymer brush surfaces. On the poly(TMAEMA) and poly(SPMA) brush surfaces, the long-range repulsive forces were observed in pure water, whereas the repulsion weakened with increase of ionic strength. Therefore, these repulsion would originate from electrostatic forces. On the poly(BMA) brush surface, the force was not observed at the approaching curve, and the strong attractive force was observed at the retracting curve in pure water. This is considered as hydrophobic interaction. On the other hand, these forces were not observed on the poly(MPC) brush surface.

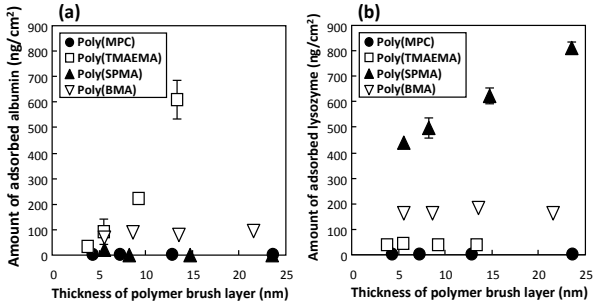

Fig. 1. Amount of adsorbed (a) albumin and (b) lysozyme on the polymer brush surfaces.

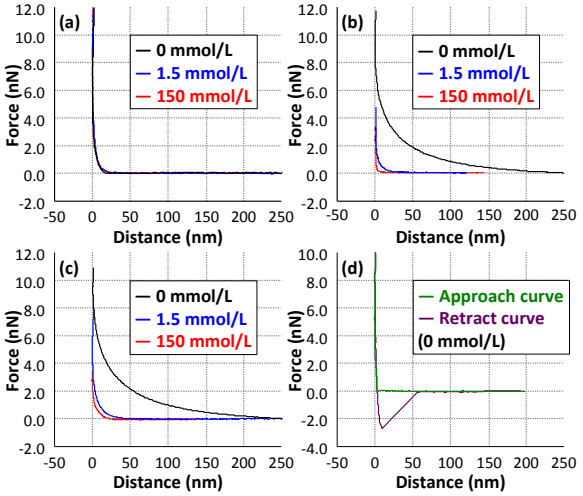

Fig. 2. Approaching and retracting curves recorded for symmetric system of (a) poly(MPC), (b) poly(TMAEMA), (c) poly(SPMA), and (d) poly(BMA) brush surfaces.

Conclusions: Strong interaction forces operated in the vicinity of the surfaces on which large amount of proteins adsorbed. Such interaction forces did not exist on the surface which suppressed protein adsorption dramatically. It was shown that the protein adsorption phenomena could be analyzed in terms of interaction forces by force curve measurement of polymer brush surfaces.

References: [1] Y. Inoue. Colloids Surf B: Biointerfaces 2010;81:350-357.