The Role of Molecular Interaction Forces in Protein Adsorption Process on Materials Surfaces

Sho SAKATA, Yuuki INOUE, and Kazuhiko ISHIHARA
Department of Materials Engineering, School of Engineering, The University of Tokyo, Tokyo 113-8656, JAPAN

Statement of Purpose: Protein adsorption is an initial and important event of biological responses which progress hierarchically at interfaces between materials surface and biomolecules. Therefore, it should be regulated completely for progress of safer regenerative medicine and advanced biomedical engineering. The objective of this study is to understand the protein adsorption process based on the molecular interacton forces generated at the surfaces. Various polymer brush surfaces were prepared using systematically selected monomers as well-characterized surfaces to clarify the interaction forces operating on the surfaces. Molecular interaction forces were evaluated by force-versus-distance (*f-d*) curve measurements of atomic force microscopy (AFM) using probes modified with various molecules. The relationship between the protein adsorption behavior and molecular interaction at the surface was discussed.

Methods: Polymer brush surfaces were prepared by surface-initiated atom transfer radical polymerization using 2-methacryloyloxyethyl phosphorylcholine (MPC, zwitterionic), 2-trimethylammoniumethyl methacrylate (TMAEMA, cationic), 3-sulfopropyl methacrylate (SPMA, anionic), and *n*-butyl methacrylate (BMA, hydrophobic) [1]. The amount of albumin (pI 4.8) and lysozyme (pI 11.1) adsorbed on the surfaces in phosphate-buffered solution (PBS; pH 7.4, ionic strength (I) = 10, 150 mmol/L) was quantified by surface plasmon resonance measurement. The *f-d* curve between the same polymer brush surfaces was recorded by AFM using probes modified with the polymer brush layers (See Fig. 2). The interaction forces between the surfaces and proteins in PBS (pH 7.4, I = 150 mmol/L) were evaluated by the AFM using protein-immobilized probe [2].

Results: Fig. 1 shows the amount of proteins adsorbed on the surfaces. The poly(MPC) surface suppressed protein adsorption regardless of ionic strength. On the poly(TMAEMA) and the poly(SPMA) surfaces, the amount of adsorbed proteins with opposite net charge was high, and the amount decreased with an increase in the ionic strength, which implies the effect of electrostatic interaction. On the poly(BMA) surface, the amount of adsorbed proteins was almost constant independent of ionic strength. Fig. 2 shows the *f-d* curves of the symmetric systems of each polymer brush surface. The poly(MPC) exhibited no specific interaction. The poly(TMAEMA) and the poly(SPMA) showed repulsion forces under low ionic strength, which would derive from electrostatic interactions. The poly(BMA) induced hydrophobic interaction after contact with the both surfaces. Such interaction force after contact was not observed in the case of other three surfaces. Fig. 3 shows the direct interaction force between the proteins and the surfaces. The data indicates good correlation against the data of Fig. 1. That is, the poly(MPC) surface did not interact with both proteins (< 1.0 nN). On the other hand, the poly(TMAEMA) and the poly(SPMA) surface strongly interacted with proteins with opposite net charge, and the poly(BMA) surface strongly interacted with both proteins. Also, these interaction forces were observed when the proteins attached at the surfaces, but were not generated during approaching process of protein toward the surface. Therefore, it is confirmed that the electrostatic and hydrophobic interactions hinder the reversible detachment of proteins from the surfaces.

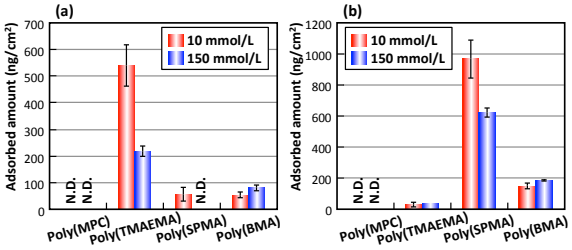

Fig. 1. Amount of (a) albumin and (b) lysozyme adsorbed on the polymer brush surfaces.

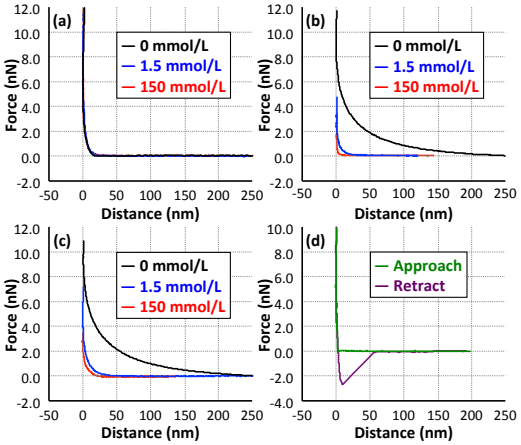

Fig. 2. Force-versus-distance curves of (a) poly(MPC), (b) poly(TMAEMA), (c) poly(SPMA), and (d) poly(BMA) brush surfaces in aqueous medium.

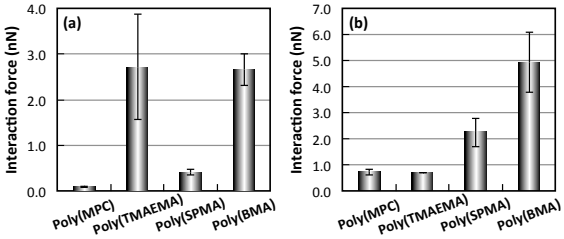

Fig. 3. Direct interaction forces between the polymer brush surfaces and (a) albumin and (b) lysozyme in PBS.

Conclusions: The electrostatic or hydrophobic interaction generating on the vicinity of protein adsorptive surfaces play a role as the force which inhibit the detachment of proteins from the surface. The fabrication of surface which enables proteins to easily detach from the surface would be important to suppress protein adsorption.

References: [1] Sakata S. Langmuir 2014;30:2745-2751. [2] Inoue Y. React. Funct. Polym. 2011;71:350-355.