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Protein adsorption and wetting of the protein adsorbed surfaces studied by a new type of laser reflectometer

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Abstract

We have devised a new type of laser reflectometer that can measure adsorption behavior of (bio)-polymers, such as proteins, on the substrate surface and also the wetting for the surface of adsorbed layer of such (bio)-polymers. The adsorption and the wetting experiments can be conducted in a sequential manner using the same sample by this apparatus. So, the wetting of the surface of protein-adsorbed layer can be measured in virtually intact state. The reflectometry is based on the traditional optical polarimetry and the wetting measurement is due to the dropping time method (DTM) that has been reported before by the authors. The two methods are combined in an apparatus and hence we can correlate the wetting of protein layer adsorbed on the substrate surfaces with the amounts of protein molecules on the surface. As a model case we demonstrate the adsorption of several typical water soluble globular proteins on stainless steel surfaces. For this combination of the adsorbent with adsorbates, it is found that the water wetting of the protein adsorbed surface is closely related with the adsorbed amounts of proteins not depending on species.

Keywords: Wetting; Protein adsorption; Reflectometry; Contact angle; Hydrophilic/hydrophobic

1. Introduction

Protein adsorption is a general phenomenon in various systems where a biological fluid contacts with any material. To date, various types of studies on the protein adsorption from solution onto surfaces have been conducted both in fundamental science and practical fields such as artificial implants in medicine or dentistry. Non-specific adsorption of protein molecules is an important event even in recent developing fields of biotechnology, e.g., bio-tips or biosensors. One kind of the studies on protein adsorption is how to prevent the adsorption of protein on surfaces, i.e., the object is how to modify the surface of materials for preventing the protein adsorption. In the process of protein adsorption, the electrostatic (or electrical double layer) and hydrophobic interactions are dominant factors in macroscopic physicochemical sense. Therefore, the control of these properties of material surface is important. Various types of surface modification have been tried for many years. Nevertheless the protein molecules can adsorb on various kinds of surfaces ranging from hydrophobic to hydrophilic ones because of hydrophobic dehydration effect of protein molecule itself at the interfacial region between protein and surface of materials. It comes from the structural feature of protein molecules, i.e., they contain hydrophobic and hydrophilic amino acid residues and they carry negative and/or positive electric charges. Hence, electrostatic and hydrophobic interactions are significant factors in the adsorption of proteins at interfaces. The water molecules that face to the hydrophobic domain of protein molecules have a tendency of leaving the surface to the bulk aqueous region. Until nowadays only some type of polymer brush on the surfaces have been found to prevent the adsorption so that the brushed surfaces are extensively studied in recent years [1–3].

Another type of study is to characterize the protein layers on the surface. Conformation, orientation, and distribution of protein molecules on the surface are keywords in this type of studies. Various types of spectroscopic studies are conduced [4].

Although the above mentioned studies have revealed the feature of protein adsorption, we have not much information on surface properties of protein layer on the substrate surfaces. The surface physicochemical properties such as electrostatic (or electrical double layer) and hydrophobic/hydrophilic ones of the protein adsorbed layer are practically important for further

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adsorption of next coming objects (molecules, cells, etc.) to the surface through the interactions between then. For example, in dentistry, it is generally believed that the first stage of formation of dental plaque is the adsorption of proteins on the surface of teeth. This phenomenon is called pellicle formation and the proceeding step involves adhesion of oral bacteria. Also, various synthetic materials are used as artificial teeth. These materials interact with saliva in the mouth and they become readily covered with saliva proteins. Oral bacteria interact with the surface of protein-covered artificial materials in the mouth. It can influence the activity of the bacteria and therewith the health condition of the human body. It is therefore important to elucidate the surface properties of the adsorbed protein layers on these materials. The hydrophobic/hydrophilic and electric properties of the outer surface of the adsorbed protein layer depend on the orientation and conformation of the protein molecules on the adsorbent surface. In other words, the kind of amino acid residues of the adsorbed protein that faces the solution side determines the surface properties in the sense of surface chemistry. So, it is valuable to measure these surface properties of adsorbed protein layers without disturbing the adsorbed state. In previous papers, we proposed the method that can measure the water wetting of a protein layer on a solid surface without crucial damage of the conformation and orientation of the adsorbed protein molecules. It is based on the measurement of the motion of a thin liquid film along the solid surface. We call this method the dropping time method (DTM). In this report, we will describe a new apparatus that can monitor the adsorption behavior in time course with succeeding detection of the wetting of protein adsorbed material surface by DTM, and some experimental results by the apparatus for typical protein molecules are shown. It is the first report that can reveal the relationship between the adsorption amounts of protein molecules and the wetting of the surface of protein adsorbed surfaces using the same sample in a virtually intact way.

2. Experimental

2.1. Measuring method and apparatus

2.1.1. Reflectometry

The adsorption behavior of proteins on the material surfaces has been monitored by a light reflectometer based on the optical polarimetry [5–8]. A linearly polarized light beam from a He–Ne laser (1 mW, Showa Optoronics Co. Ltd., Japan) is passed through a polarizer prism of which orientation of polarization is at 45° to a sample plate, which gives an equal light intensity to parallel (Rp) and perpendicular (Rs) components of the laser beam against the incident plane. The incident light angle and reflected one can be scanned automatically from 45° to 85° against the sample plate. The reflected light is divided to two components through a beam splitting prism and both intensities of the components are detected by two silicon photodiodes (0.58A/W, S2387-1010R, Hamamatsu Photonics Co. Ltd., Japan). The electrical signals converted from the light intensity by photodiodes are led to an electric amplifiers and then to a notebook computer (PC-LG26SUMMF, Windows Xp, Pentium 4, NEC Co. Ltd., Japan) through an A/D converter (CBI-3133A, 12 bits, 10 μs, Interface Co. Ltd., Japan). The motorized stage (CSG-602R, 12.5 k pps, 2.5°/10000 pulses, Sigma Koki Co. Ltd., Japan) to change the light incident angle is controlled by the computer using a communication method called the RS232C standard. Most of other optical parts were purchased from Sigma Koki Co. Ltd., Japan. The optical constants: refractive index (n) and absorption coefficient (k) of substrate material were determined by the measurements of the dependency of the ratio, Rp/Rs, on the light incident angle in air. The analyzing program is based on the simple three layer model of reflection/refraction optics. The experiments of protein adsorption were conducted utilizing a solution cell (Fig. 1a). In this case the incident light angle was set at 75° to get rather high signal ratio (near the Brewster angle). The adsorption experiment was started by replacing pure water in the vessel cell by protein aqueous solution of certain concentration. The aqueous solution in the cell was continuously stirred by a magnetic stirrer with a tip. The cell has two water inlets/outlets at upper and lower side. The ratio of Rp/Rs was continuously monitored against elapsed time. The change in Rp/Rs can be related to the thickness (d) and the refractive index (n_p) of the adsorbed protein layer on substrate surface. The adsorption amount per unit area (Γ) can be calculated utilizing the equation: $\Gamma = (n_p - n_w)d/(dn/dC)$, where n_w is refractive index of aqueous solution (1.33), dn/dC is the dependence of refractive index of protein aqueous solution on protein concentration, and others are mentioned before.

2.1.2. Wetting measurements (dropping time method)

The hydrophobic/hydrophilic character of a surface can be probed by its water wetting, which is usually assessed by conducting the contact angle measurements utilizing a sessile drop of water or an air bubble on the surface that is immersed in water, etc. However, protein molecules adsorbed on surfaces easily change their conformation on drying, so there is high possibility that we measure the contact angle for denatured protein layer by the sessile drop method. Or in another situation, the water molecules at three phase contact line of a water drop interact with two kinds of surfaces, i.e., one is natural protein layer at solid/liquid interface and the other is denatured protein layer at solid/gas interface by the sessile drop method. Therefore, it is not confirmed that the measured values reflect the surface as it is when immersed in an aqueous environment. So, we have devised a method that can measure the wetting in a virtually intact situation. It is called dropping time method. The basic concept of this method and also some experimental examples were described in previously published papers [9–12]. Briefly, to detect the wetting of the surface of a protein layer adsorbed on a solid substrate, the motion of a thin liquid film dropping along the protein layer is measured by means of light reflection of laser beams. Typically, two laser beams are impinged on two positions separated by X distance on the solid surface and the reflected light beams are detected by two photo-detector diodes, respectively. A vessel cell containing a protein solution is mounted below the vertically suspended sample plate. By ascending the vessel cell, the sample plate is immersed into the solution until the two light beams are under the air/protein solution interface in the vessel.