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Properties of modified surface for biosensing interface

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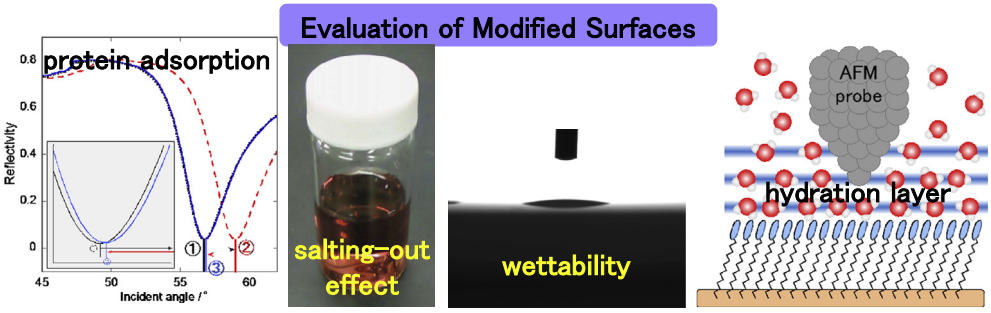
GRAPHICAL ABSTRACT

In this work, properties of modified surface with various mercapto compounds were evaluated in point of protein adsorption, salting-out effect, wettability, and hydration layer.



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ABSTRACT

Properties of modified surface, behavior against salting-out effect, suppressive effect for protein nonspecific adsorption, and wettability were examined using various mercapto compounds bearing methyloligoethylene glycol, oligoethylene glycol, alkyl oligoethylene glycol, alkyl phosphoryl choline, alkyl inverse phosphoryl choline, and alkyl sulfobetaine moieties. The behavior against salting-out effect was examined using gold nanoparticle with PBS and NaCl aqueous solution. The suppressive effect for protein nonspecific adsorption was evaluated by SPR, and the wettability was measured on the SPR chip. The gold nanoparticle modified with 8C3EG, 12C4EG, 12CPC, 6CCP, and 12CCP showed excellent behavior against salting-out effect. The suppression of protein nonspecific adsorption was effective with 6EG, 12C4EG, 12CPC, and 12CS. On the other hand, the modified surface possessed high wettability except for the surface modified with M6EG. The results indicate that incorporation of alkyl group into surface modification materials is effective for the enhancement of behavior against salting-out effect and suppressive effect for protein nonspecific adsorption regardless of wettability. Among the zwitter ionic derivatives, inverse phosphoryl choline derivatives showed intriguing properties, high behavior against salting-out effect with high wettability but low suppressive effect for protein nonspecific adsorption.

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1. Introduction

Properties of modified surface for biosensing interface are recognized as important subjects to develop methodologies in bio-related analysis and techniques. Therefore, various surface modification materials to fabricate biosensing interface have been synthesized and studied on properties of modified surface for application to biosensor and carrier of drug delivery system. As an evaluation indicator for modified surface, wettability is known as a traditional scale to represent surface properties for bio-related application, especially in surfaces coated with polymers. Generally, it is considered that surface with higher wettability affords superior biosensing interface to show suppressive effect for protein nonspecific adsorption and behavior against salting-out effect [1,2]. These two properties are of great interest because they are essential to develop biosensor and carrier of drug delivery system. However, the relationship among wettability, suppressive effect for protein nonspecific adsorption, and behavior against salting-out effect is ambiguous, and there are few systematic studies using various surface modification materials for comparison.

In the biosensor development, various systems have been studied to detect biomarkers such as proteins, hormones, and carbohydrates, as those biomarkers are reported to be useful for diagnosis [3,4]. When measuring biomarkers, it is well known that nonspecific adsorption of non targeted proteins contained in samples to biosensing interface can cause serious problem, where the nonspecific adsorption induces undesirable response of biosensing interface and high background noise resulted in deterioration of sensor performance [5–8]. Therefore, for fabrication of a desirable biosensing interface, development of surface modification materials to suppress nonspecific adsorption of proteins is recommended [9–12], and then, incorporation of functional materials such as antibody and enzyme into the biosensing interface is essential [13–15]. On the other hand, construction of drug carriers is one of fundamental technologies in drug delivery system. For drug carriers, behavior against salting-out effect is commonly required, as the drug carriers are usually particle containers, which are supposed to circulate in body fluid. In addition, suppressive effect for protein nonspecific adsorption is also recommended in order that carriers come at target part precisely [16–21].

In order to obtain systematic insight about surface modification materials, we synthesized various mercapto compounds bearing methyloligoethylene glycol, oligoethylene glycol, alkyl oligoethylene glycol, alkyl phosphoryl choline, alkyl inverse phosphoryl choline, and alkyl sulfobetaine moieties, and studied surface properties modified with those materials in this work. As evaluation indicators for surface properties, behavior against salting-out effect, suppressive effect for protein nonspecific adsorption, and wettability were examined and compared. In the study of behavior against salting-out effect, gold nanoparticle (15 nm in diameter) was employed, and the behavior to salting-out effect for the surface-modified gold nanoparticle was examined in the presence of phosphate buffered saline (PBS) or NaCl (ca. 2.5 M). Suppressive effect for protein nonspecific adsorption was evaluated by SPR (surface plasmon resonance) measurement, and wettability was measured on the same surface used for SPR measurement, namely, on the surface of SPR chip. In this study, we adopted human serum for the measurements of suppressive effect for protein nonspecific adsorption because it is reported that nonspecific adsorption of proteins is induced by not only interaction of a protein with surface but also interaction among proteins to adsorb onto surface [22]. The wettability was also measured using human serum. On the other hand, it has been reported that properties of surface, especially suppressive effect for protein nonspecific adsorption is depending on hydration layer of interface [23]. Therefore, we examined hydration layer on the modified gold surface by the force-mapping method with atomic force microscope (AFM) [24–26]. The obtained data should be instructive for fabrication of high-performance biosensing interface with mercapto compounds.

2. Experimental section

2.1. Materials and methods

All chemicals were commercially available and used as received without additional purification. Details of synthesis procedures are described in Supplementary material. Gold nanoparticle colloid solution (15 nm diameter coated with citric acid in water) was purchased from Tanaka Kikinzoku Kogyo Japan. The gold nanoparticle is synthesized by reductive method and single crystal-like. The SPR chip was prepared radio frequency sputtering, and the surface is polycrystalline. Control serum based on human serum (No. 717438) was purchased from Roche Diagnostics K. K. Japan. All measurements were reiterated more than several times to show average values.

2.2. Evaluation for behavior against salting-out effect

For surface modification of gold nanoparticle, mercapto compound solution with ethanol (100 mM, 0.1 mL) was added dropwise to the gold nanoparticle colloid solution (0.9 mL) under vigorous stirring at room temperature. The gold nanoparticle solution was stood for over night, and irradiated ultrasound for 1 min before use. To the gold nanoparticle solution, equal volume of twofold-concentration PBS (without magnesium and calcium) or saturated NaCl aqueous solution was added dropwise under vigorous stirring at room temperature. The UV–vis spectra for the gold nanoparticle solutions were measured for comparison.

2.3. Measurement for molecular concentration of SAM

The SAMs were fabricated on the surface of clean single crystal gold by using 0.1 mM thiols in ethanol - water (50 vol.%). Immersion time was 10 min. The modified surface was rinsed with ethanol, and then, with water several times. The electrochemical reductive desorption method on gold surface was adopted to determine molecular concentrations of membranes [27]. Cyclic voltammetry was carried out in 1 M KOH solution at room temperature, using a PAR 263A (EG&G Princeton Applied Research) potentiostat equipped with an external potential scanner (model 175, EG&G PARC). The sweep rate was 20 mV/s and the electrolyte solution was deaerated by flowing Ar gas. Saturated calomel electrode (SCE) and Pt wire were used for the electrochemical measurements as reference and counter electrodes, respectively.

2.4. Measurement for nonspecific adsorption of proteins by SPR

Gold-coated LaSFN9 glass chip was prepared to evaluate the amount of protein nonspecific adsorption by SPR method. Prior to metal coating, the chip was cleaned by sonication in a 1% Hellmanex solution. Cr and Au films were deposited by rf-sputtering onto the chip. The film thickness was less than 1 nm and 47 ± 3 nm for Cr and Au film, respectively. And then, an ethanol - water (50 vol.%) solution (2 mM) of surface modification materials was put onto the chip surface, and the chip was stood for 2 h at room temperature. After rinsing with ethanol - water (50 vol.%), the chip modified with SAM was dried and covered with a cover glass using a double-sided adhesive tape. The SPR optical setup is described in the previous work in detail [28]. A He-Ne laser light operating at 632.8 nm (Meles Griot) was passed through an optical chopper, polarizer, and analyzer. The light reflected at the chip