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Optimization of DNA-directed immobilization on mixed oligo(ethylene glycol) monolayers for immunodetection

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ABSTRACT

The development of protein chips has suffered from problems regarding long-term protein stability and activity. We present a protein sensor surface for immunodetection that is prepared by a DNA-directed protein immobilization method on a mixed self-assembled monolayer (SAM). By this approach, an immobilized single-stranded DNA (ssDNA) surface can be transferred/modified into a protein chip by flowing in ssDNA-conjugated protein when the protein chip measurement is needed. Therefore, the long-term stability of the protein chip will not be a problem for various applications. We tried various compositions for the SAM layer, the length of the ssDNA spacer, the end-point nucleotide composition, and the processes of ssDNA immobilization of the SAM for an optimized condition for shifting the DNA chip to a protein chip. The evaluations were made by using surface plasmon resonance. Our results indicated that a 50:1 ratio of oligo(ethylene glycol) (OEG)/COOH-terminated OEG and DNA sequences with 20 mer are the best conditions found here for making a protein chip via a DNA-directed immobilization (DDI) method. The designed end-point nucleotide composition contains a few guanines or cytosines, and ssDNA immobilization of the SAM by dehybridizing immobilized double-stranded DNA (dsDNA) can improve the hybridization efficiency.

Physical adsorption at a solid surface, cross-linking between molecules, covalent binding to a surface, entrapment within a membrane, and surfactant matrices, polymer, or microcapsules are all commonly used immobilization methods on the transducer surface of biosensors [1,2]. Biosensors are classified into immunochemical, enzymatic, and nonenzymatic receptors, whole-cell, and DNA biosensors according to the biorecognition principle [2]. Immunoassays require immobilizing one of the antibodies onto a surface. The simplest way to immobilize antibodies onto a sensor surface is with noncovalent adsorption. However, this type of procedure has several disadvantages such as denaturing the majority of adsorbed proteins, random orientation, and nonspecific protein binding [3]. Antibodies can be biotinylated and then immobilized onto streptavidin-coated surfaces [4], or they can be directly coupled to amine-reactive surfaces [5–7]. These methods reduce the denaturing of immobilized antibodies, but some of the immobilized protein may lose its binding activity due to steric hindrance, direct chemical modification of the antigen binding site, and strain from multiple attachment sites [3,8]. Some studies demonstrate that antibodies are immobilized with a proper orientation so that the binding site of the antigen is exposed to the solution phase and presents superior antigen binding capabilities [9,10]. Owing to the oriented immobilization of antibodies being crucial for immunodetection, other strategies are applied to provide a more orderly immobilization of antibodies on the sensor surface. An effective approach for immobilizing the specific antibody, namely immunoglobulin G (IgG),[1] with correct orientation on the surface plasmon resonance (SPR) sensor surface is to use binding proteins such as proteins A and G [11–15]. Proteins A and G have Fc binding

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[1] *Abbreviations used:* IgG, immunoglobulin G; SPR, surface plasmon resonance; ssDNA, single-stranded DNA; OEG, oligo(ethylene glycol); SAM, self-assembled monolayer; EG, ethylene glycol; DDI, DNA-directed immobilization; ESCA, electron spectroscopy for chemical analysis; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; sulfo-NHS, *N*-hydroxy-sulfo-succinimide; sulfo-SMPB, sulfo-succinimidyl 4-(*p*-maleimidophenyl) butyrate; HSA, human serum albumin; TCEP, tris(2-carboxyethyl)phosphine hydrochloride solution; TFA, trifluoroacetic acid; PBS, phosphate-buffered saline; SP, surface plasmon; MWCO, molecular weight cutoff; dsDNA, double-stranded DNA; DI, deionized; BSA, bovine serum albumin.

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domains that can interact specifically with the Fc portion of the IgG. DNA-directed protein immobilization is another useful method that can immobilize protein–DNA conjugates on the surface via sequence-specific hybridization that can provide a well-ordered orientation of immobilized antibodies [16,17]. Furthermore, the tailored surface composition of single-stranded DNA (ssDNA)/oligo(ethylene glycol) (OEG) self-assembled monolayers (SAMs) can provide an optimal interaction for DNA hybridization.

The pairing of complementary DNA sequences is applied in a variety of biological techniques. Sequence and quality of DNA, hybridization temperature, hybridization buffer (ionic strength), agitation rate, hybridization time, concentration of DNA, surface charge, and linker length and flexibility are factors that can affect DNA hybridization [18]. During an ideal hybridization process, the ssDNAs will pair under complementary probe sequences on a solid support. The intensity of the measured signal can then be translated directly into the relative quantities of paired DNAs. However, ssDNA oligomers possessing self-complementary sequences may form hairpin (i.e., stem–loop) or duplex secondary structures. DNA hairpin or duplex structures have been found to be relatively stable and to interfere with the normal hybridization process [19,20]. Careful design or selection for the pairing of complementary DNA sequences in the DNA hybridization experiments is critical. Boozer and coworkers [16,17] demonstrated a feasible method for using DNA-directed assembly to convert an ssDNA probe surface into a protein biosensor in one simple step by using DNA–protein conjugates. The conjugate coverage on the sensor surface was influenced by the DNA probe density and probe sequence. Different probe sequence candidates showed different amounts of SPR response to complementary hybridization [17]. To accomplish a greater effect of protein immobilization through a DNA-directed method, the complementary DNA sequences need to be chosen to ensure that the DNA sequences have superior hybridization efficiency.

SAMs of alkanethiolates have typically been used to prepare patterned sensor surfaces in biosensing. These SAMs may be used as a linker layer for immobilizing biological components at the transducer surface or used directly to study protein adsorption and cell adhesion [21,22]. Harris [23] proposed that alkanethiols with the ethylene glycol (EG) group could prevent the adsorption of protein. The SAMs formed by alkanethiols with short oligomers of the ethylene glycol group [$(OCH_2CH_2)n$OH, which can also be expressed as (EG)nOH, $n = 3–6$] exhibit a good performance in preventing the adsorption of proteins [22–24]. In experiments that studied cell adhesion, EG2OH- to EG6OH-coated surfaces have not inhibited spore settlement. Nevertheless, the adhesion between spore and surface was minimal and the surfaces are referred to as adhesive-resistant surfaces [25]. The mechanism of surface resistance to the adsorption of proteins has been widely accepted as water at the protein–OEG interface in resisting nonspecific protein adsorption [26–30].

Researchers found that OEG SAMs can resist nonspecific protein adsorption within a certain range of the normalized surface density of OEG chains (the ratio is ~0.6–0.8) and proteins that can adsorb on the OEG-coated surface when density of the OEG chains is too high or too low [31]. The simulation results indicated a larger number of tightly bound water molecules around OEG chains at reasonable OEG densities [32]. The simulation and experimental results indicated that there is a correlation between the OEG surface resistance to protein adsorption and the hydration of OEG chains [31,32]. OEG SAMs are currently the most effective inert surface chemistry and have been widely used in the biomedical field for many years [23]. They are also used in the preparation of sensor surfaces for biosensing [16,17,33–35]. A mixture of OEG and COOH-terminated OEG is currently used to functionalize chips prior to forming a mixed OEG SAM that can provide a nonfouling background and anchor biomolecules by amino coupling [34–36]. The nonfouling background can reduce the false detections that are primarily caused by the nonspecific binding of proteins, and this is important for developing sensors. In these aforementioned studies, antibodies were immobilized by being covalently linked on chemically activated solid surfaces.

In this study, a protein chip was created for SPR measurement through DNA-directed immobilization (DDI) on the mixed OEG/COOH–OEG SAM. The probe DNA was immobilized on the mixed OEG/COOH–OEG SAM by amino coupling and hybridized with its complementary sequence conjugated with an antibody. The mixed OEG/COOH–OEG SAM and the immobilization of ssDNA on the sensor surface were examined by electron spectroscopy for chemical analysis (ESCA). The properties of protein adsorption resistance on the sensor surfaces are composed of different DNA sequences and SAMs formed by various molar ratios of OEG/COOH–OEG that were examined. This investigation endeavored to find an optimum among the length of the conjugate ssDNA, the end-point sequences of the ssDNA spacer, the process of the immobilization of the ssDNA spacer, and the molar ratio/composition of OEG/COOH–OEG of the sensor surface.

Materials and methods

Materials

1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), *N*-hydroxy-sulfo-succinimide (sulfo-NHS), and sulfo-succinimidyl 4-(*p*-maleimidophenyl) butyrate (sulfo-SMPB) were purchased from Thermo Scientific (USA). Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2\ H_2O$), and sodium hydroxide were purchased from Merck (Germany). Sodium chloride, human serum albumin (HSA), monoclonal anti-human serum albumin (anti-HSA), tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP), trifluoroacetic acid (TFA), absolute ethanol (99.5%), ethanolamine, and lysozyme (from chicken egg whites) were purchased from Sigma–Aldrich (USA). Anti-lysozyme (from hen eggs) was obtained from AbD Serotec (UK). OEG-terminated thiols [$HSC_{11}(EG)_6OCH_2COOH$ and $HSC_{11}(EG)_6OH$] were purchased from ProChimia (Poland). Phosphate-buffered saline (PBS) tablets were acquired from Amresco (USA); these are convenient for the preparation of 1× PBS solution, which contains 10 mM phosphate buffer, 137 mM sodium chloride, and 2.7 mM potassium chloride (adjusted to pH 7.4). All other chemicals used in this study were reagent grade.

ESCA measurements

ESCA has a high resolution to determine the composition or performance of advanced materials. In this study, ESCA was used to verify the outcomes of surface modifications on sensor surfaces. These measurements were performed on a Thermo VG Scientific–Sigma Probe system (Thermo Fisher Scientific, USA) in the Precision Instrument Center at National Central University (Taiwan). The Sigma Probe can provide high-sensitivity analysis from small areas (down to 15 μm). The SPR chips were used directly to prepare samples for ESCA measurements.

SPR measurements

In this study, we used an SPR platform to perform all experiments. The SPR sensor platform was developed based on spectroscopy [37], and surface plasmons (SPs) in the instrument are excited by the attenuated total reflection (ATR) method based on the Kretschmann configuration. A collimated polychromatic light