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Preparation of a surface-grafted protein-selective polymer film by combined use of controlled/living radical photopolymerization and microcontact imprinting

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ABSTRACT

In this study, we describe a strategy for the formation of a molecularly imprinted polymer (MIP) capable of selective rebinding of protein-sized molecules and interfaced with a planar sensing surface. The strategy is based on the synergistic use of the surface-initiated controlled/living radical (C/LR) photopolymerization and microcontact imprinting approach aiming at design of a protein-responsive polymer for biosensing application. Bovine serum albumin (BSA), 2-(diethylamino)ethyl methacrylate, bis-acrylamide were used as a model protein, a functional monomer and a cross-linker, respectively, to prepare the BSA-MIP film. The optimal parameters of C/LR photopolymerization such as the method for photoinitiator attachment to the sensor surface, monomer:cross-linker molar ratio, polymerization time, were determined. The BSA-MIP film were studied in terms of their recognition capability and selectivity towards the target protein (BSA) through the analysis of the responses of the BSA-MIP modified SPR sensors upon interaction with BSA and interfering proteins, human serum albumin (HSA) and Fc-fragment of immunoglobulin G (Fc). It was found that BSA-MIP adsorbed BSA with the dissociation constant (KD) in the nanomolar range (68 nM) and shows more than two times higher adsorption capacity as compared to HSA and Fc, even though their molecular sizes were similar. Also, BSA-MIP could be perfectly regenerated in the alkaline solution showing nearly reversible responses (loss of 2.4%) even after the 25th regeneration cycle. The presented simple synthesis strategy could be potentially employed for the preparation of protein-MIP films on a planar sensor transducer allowing to develop sensing systems for detection of clinically relevant proteins.

1. Introduction

The concept of molecular imprinting has been widely recognized as a promising strategy for synthesis of robust molecular recognition materials with high selectivity towards the analyte [1]. Molecular imprinting can be defined as the process of template-induced formation of specific molecular recognition sites in a polymer matrix material. In this process, a mixture of functional monomers is polymerized around a chosen target molecule acting as a template. Removal of the templates from the formed polymer leaves behind binding sites that are capable of selectively recognizing of the template molecules or similar structures. The main benefits of these, so-called Molecularly Imprinted Polymers (MIPs), are related to their synthetic nature, i.e., excellent chemical and thermal stability associated with reproducible, cost-effective fabrication. Therefore, MIPs have received much interest and are successfully applied in different areas such as artificial antibody mimics [2], materials separation [3] and drug delivery [4]. MIPs have been reported to be a promising alternative to biological receptors in biosensors providing more stable and low-cost recognition elements [5]. Bulk imprinting is the simplest approach to macromolecular imprinting. The advantage of this approach is that the 3D–binding sites are formed for the whole protein. However, a significant disadvantage is the diffusional limitations, as the polymer matrix surrounding the protein from all sides and prevents protein washing from the matrix [2]. To solve this problem Shi and coworkers proposed to precipitate the protein on the surface of mica before the polymerization [6]. Thus, the growth of the polymer matrix is limited to the surface of protein stamp and the resulting specific cavities are located very close to the surface of the polymer. Nowadays, surface imprinting of proteins has become the most common used approach [7–9]. One of the prospective approaches to produce protein-MIP films with surface-confined binding sites is microcontact imprinting [10,11]. An important advantage of the

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microcontact imprinting is that very little or no template molecules remain "trapped" in the polymer matrix after completion of the polymerization resulting in a MIP-film with more homogeneous binding sites [12].

In addition to the above mentioned techniques, the development of MIPs for biosensing application requires a perfect interfacing between the recognition element and the sensor transducer. Popular methods of polymer film preparation on a solid surface such as spin-casting, dip-coating, polyelectrolyte deposition and plasma deposition result in physically adsorbed polymer films, which exhibit non-covalent interactions (hydrophobic or electrostatic interactions, van der Waals forces) with the surface. This gives a relatively weak adhesion, making these films not steady to external factors (solvents, high temperature, mechanical actions) and limits the scope of their application [13]. Surface initiated polymerization is an universal way to overcome these disadvantages by creating covalent bonding between the polymer film and the surface [14]. To perform surface initiated polymerization an initiator is firstly attached to the substrate surface using different techniques [14,15]. This method offers the capability of the film thickness control, uniform coating of surface, control over composition, and high density of grafting [14]. Surface initiated polymerization can proceed via different polymerization mechanisms depending on the initiator end-group [16]: free-radical polymerization (FRP) [17], cationic and anionic polymerization [18], atom-transfer radical polymerization (ATRP) [19], ring-opening polymerization [20] and reversible addition fragmentation chain transfer (RAFT) polymerization [21].

Photopolymerization seems to be suitable methods for in situ MIP film synthesis providing the possibility of good control of both film thickness and inner morphology [22]. A wide range of photoinitiators allows realizing photopolymerization by various mechanisms, including the controlled/living radical polymerization (C/LRP) method, which allows the control of the composition and thickness of the MIP films. Salian and co-workers showed the improvement in network homogeneity and imprinting efficiency in weakly cross-linked MIP networks prepared by C/LRP in comparison with FRP [23]. C/LRP extended the reaction-controlled regime of the polymerization reaction and formed more homogeneous polymer chains and networks with smaller mesh sizes. The RAFT polymerization is the most versatile process among C/LRP techniques. Effective chain transfer agents for the reversible attachment mechanism fragmentation are different dithioesters, dithiocarbamates, trithiocarbamates, and xanthates [24]. RAFT process initiators are called iniferters (initiator–transfer agent–terminator) [25]. The RAFT mechanism is widely used for the synthesis of MIP-particles [26–30], MIP-fibers [31] as well as MIP films directly on the sensor surface [32,33]. RAFT approach has been successfully applied for imprinting of small molecules [23,29] as well as macromolecules such as proteins [34].

The aim of the study is to develop a strategy for the preparation of a protein-imprinted polymer film (protein-MIP) directly on the surface of a planar sensor by the combination of the controlled/living radical photopolymerization initiated from the surface and microcontact molecular imprinting. Bovine serum albumin (BSA), 2-(diethylamino)ethyl methacrylate, bis-acrylamide were used as a model protein, a functional monomer and a cross-linker, respectively to prepare the BSA-MIP films. The optimal parameters of C/LRP, such as the method for photoinitiator attachment to the sensor surface, monomer/cross-linker molar ratio and polymerization time, were determined. The prepared BSA-MIP films were studied in terms of their recognition capability and selectivity towards the target protein (BSA) through the analysis of the responses of the BSA-MIP modified SPR sensors upon interaction with BSA and interfering proteins, human serum albumin (HSA) and Fc-fragment of immunoglobulin G (Fc).

2. Experimental

2.1. Chemicals and materials

3,5-dichlorophenyl diazonium tetrafluoroborate (3,5-DClPDT), sodium diethyldithiocarbamate (NaDEDTC), acetonitrile (ACN), (3-glycidyloxypropyl)trimethoxysilane (3-GPS), *N*,*N*′-methylenebis(acrylamide) (BAA), diethylaminoethyl methacrylate (DEAEM), bovine serum albumin (BSA, 66 kDa), human serum albumin (HSA, 66.5 kDa) and sodium dodecyl sulfate (SDS) were obtained from Sigma-Aldrich. Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4, ⩾99.0%,) was obtained from Fluka. Fc-fragment of immunoglobulin G (Fc, 50 kDa) was obtained from Icosagen AS (Estonia). All chemicals were of analytical grade or higher and were used as received without any further purification. *para*-Maleimidophenyl diazonium tetrafluoroborate (p-MPDT) was synthesized from *N*-(4-aminophenyl)maleimide (TCI Europa) and identified by nuclear magnetic resonance spectroscopy in CD_3-CN (8.55 doublet, 2H; 8.12 doublet, 2H; 7.07 singlet, 2H). Ultrapure water (resistivity 18.2 MΩ·cm, Millipore, USA) was used for the preparation of all aqueous solutions. Phosphate buffered saline (PBS) solution (0.01 M, pH 7.4) was used to prepare analyte solutions.

2.2. Synthesis of BSA-MIP

The BSA-MIPs were synthesized directly on the SPR sensor surface using the following main stages (Fig. 1): (a) covalent immobilization of BSA on the surface of a glass slide via 3-GPS, (b) modification of the gold surface of the sensor by the iniferter, DEDTC, and coating by the mixture of the functional and crosslinking monomers (DEAEM and BAA) (c) bringing the BSA-modified glass slide into contact with the DEDTC-modified SPR sensor and initiation of polymerization by UV-irradiation, (d) detaching the glass slide from the SPR sensor leaving behind BSA imprints on the polymer surface.

2.2.1. Immobilization of BSA on a glass slide

A cover glass slide (22 × 22 mm) was cleaned in a base piranha solution (3:1 volume ratio of 30% NH_4OH and 30% H_2O_2) for 30 min with heating up to 60 °C and rinsed abundantly with ultrapure water. The cleaned glass slide was transferred to ethanol for 10 min and then dried with a nitrogen flow. The glass slide was immersed in 5% 3-GPS chloroform solution for 30 min. After the reaction, the glass slide was rinsed by chloroform to remove any unbound 3-GPS molecules and dried with nitrogen and stored in an oven at 180 °C for 2 h. Finally, the epoxy-silane modified glass slide was incubated in PBS buffer solution containing 0.05 mg/ml BSA for 2 h at room temperature, washed with water and dried under nitrogen flow. Quality of protein immobilization on the glass slide was evaluated by epifluorescent microscopy and contact angle (CA) measurements. The epifluorescent imaging was performed via a 20× plan fluorite objective (Plan Fluor Epi BD, NA0.45, WD4.5 mm) using a Peltier-cooled charge-coupled device camera (DS-5Mc) attached to a research microscope (Eclipse LV100D, Nikon Instruments, Japan) equipped with a fluorescence illumination system (Lumen 200, Prior Scientific Inc., USA). NIS-Elements imaging software was used to analyze the fluorescent images calculating the mean pixel intensity in a region of interest. CA measurements were performed at room temperature using a drop shape analyzer (DSA25, Krüss GmbH, Germany). A drop of water (7 μl) was semi-automatically placed onto the polymer surface using Hamilton syringe and the contact angles indicating the wetting ability of the materials were calculated automatically.

2.2.2. Modification of the gold surface of the sensor by the iniferter

The gold surface of SPR sensor served as a substrate for iniferter (DEDTC) grafting. The sensor surface was treated by an UV ozone cleaner (PSD Pro Series, Novascan Technologies, Inc., USA) for 30 min followed by rinsing with ultrapure water and drying with a nitrogen