Synthetic Strategies of Selective Biomimetic Polymer for Human Serum Albumin

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Abstract:
Molecularly imprinted polymer (MIP) has become an outstanding selective element of clinically relevant analytes. In this study, we present a biomimetic surface imprinting strategy for human serum albumin (HSA), which can be found in both sera and urine. To begin with, the composition of the polymer was synthesized using the starting point of previously published MIP for bovine serum albumin (BSA). Both HSA and BSA have closely related properties in terms of size (14×4×4 nm) and heart-shaped molecule, but differ in the number of amino acids (585 amino acids of HSA, 583 amino acids of BSA), and also in types of amino acids (145 non-identical amino acids). HSA-MIP, comprising co-polymers of acrylic acid and N-vinylpyrrolidone at the ratio of 2:3 crosslinked with ethylene glycol dimethacrylate, were screened on 10 MHz dual-electrode quartz crystal microbalances (QCM) for binding ability investigation. Such MIP yields much higher sensor response towards 3 mg/mL of HSA concentration (520 Hz) than other urinary substances at ten-time higher physical relevant concentrations (73 Hz of 0.13 mg/mL creatinine, 0 Hz of 2.5 mg/mL cortisol). It indicates that MIP favors HSA very strongly and can be used further to develop device for albumin determination in urine.

1. Introduction

While biological receptors, such as antibodies, DNA, hormone receptors, and enzymes offer satisfactory to excellent affinity and specificity, they are often affected by limited stability, poor performance in non-aqueous media, poor reproducibility, and high production costs. Under these circumstances a group of synthetic materials with binding abilities comparable to natural receptors, namely molecularly imprinted polymers (MIPs), have become increasingly important as biomimetic receptors. MIPs are synthesized via molecular imprinting. It results in a polymer containing specific recognition sites that are complementary to the template in size, shape, and positioning of functional groups.¹ Figure 1 shows the schematic of MIPs synthesis. First an interaction network is pre-formed between (functional) monomer(s) and a template species in the presence of cross linker through covalent or non-covalent interactions. The latter is usually the analyte-to-be or a suitable model compound. The second step is polymerization by photochemical or thermal initiation. After template removal, the polymer network reveals cavities that are complementary to the respective template.

Figure 1. Principle of MIP synthesis
MIPs efforts have been reported in a broad range of potential areas such as separation sciences, bio/chemical sensors, catalysis, drug delivery. To date, MIPs have been synthesized successfully for wide a range of analytes from small organic molecules (e.g. pesticides, pharmaceuticals, and nucleic acids), to large bio (macro) molecules (e.g. proteins, and cholesterol). However, imprinting approach of such large biochemical compounds is still a challenge because they are flexible and also exposed a large number of functional groups on their surfaces. An imprinting model of protein, namely albumin, which is the principal component of blood plasma for maintaining colloid osmotic blood pressure, has been widely studied. Albumin in the urine is also used as a screening biomarker for renal disease and diabetic mellitus in human. In recent years, bovine serum albumin (BSA) were successfully studied to find out the optimized protocol for generating selective polymer. However, human serum albumin (HSA) offers advantages over BSA in real-life clinical practice. Albumins from different mammalian species of bovine and human reveal dissimilarities in the physio-chemical properties. Hence, polymer optimization turned out one of the main challenges between both proteins. In this work, we present MIP synthetic strategies of HSA for biomedical applications e.g. using HSA-MIP as the biomimetic recognition element of the sensor system.

2. Materials and Methods
2.1 Chemicals
Human serum albumin (HSA, lyophilized powder), N-vinylpyrrolidone (VP), ethylene glycol dimethacrylate (EGDMA), and 2,2′-azobis(isobutyronitrile) (AIBN) were obtained from Sigma-Aldrich (Steinheim, Germany). Acrylic acid (AA), magnesium chloride (MgCl₂), sodium chloride (NaCl), and acetic acid were purchased from Merck (Darmstadt, Germany). Brilliant gold paste (gold colloid, 12% gold content) was purchased from Heraeus, Germany. All reagents were analytical or highest synthetic grade commercially available.

2.2 Apparatus
Brilliant gold paste (Heraeus; 12%) was screen-printed to establish dual gold-electrode patterns onto 10 Megahertz (MHz) QCM as shown in Figure 2, yielding QCM transducer.

Figure 2. QCM with dual-electrode configuration

2.3 Synthesis of HSA-MIP
HSA and BSA have closely related properties in terms of size (14×4×4 nm) and heart-shaped molecule, but differ in the number of amino acids (585 amino acids of HSA, 583 amino acids of BSA), and also in types of amino acids (145 non-identical amino acids). Moreover, these two albumins are different in terms of surface hydrophobicity properties, electrophoretic behavior, thermal and chemical stability. HSA-MIP was firstly synthesized followed the BSA-MIP recipes, comprising copolymers of acrylic acid and N-vinyl pyrrolidone (ratio at 2:3) crosslinked with ethylene glycol dimethacrylate (EGDMA). This solution was pre-polymerized under UV for 20 min approaching the gel state, which is proper for spin-coating to yield a thin-film on the electrodes of QCM. Then, the HSA template stamp was pressed into the pre-polymer film on the first electrode of QCM to yield the MIP. The polymer on the untreated side of the electrode leads to so-called non-imprinted polymer (NIP) using as reference electrode. Then, the QCM was placed in an oven to complete polymerization. After that, the HSA was
washed out using 10% aqueous solution of acetic acid, followed by 0.1% SDS solution, and deionized water, yielding HSA-MIP QCM sensor as shown in Figure 3.

**Figure 3.** Schematic of the HSA-MIP QCM sensor

2.4 Testing binding ability of HSA-MIP via QCM

The sensor operated at 25 °C with the QCM in measuring cell. Baseline signals were obtained via 10 mM phosphate buffer saline (PBS), pH 7.4, until the equilibrium state was reached. Then, the binding ability of HSA to the recognition cavities on HSA-MIP was determined by exposing the sensor system to standard HSA solutions at the concentration of 3 mg/mL.

2.5 Testing of selectivity for HSA-MIP

The sensor was exposed to each standard solution comprising of 3 mg/mL of HSA, 0.13 mg/mL of creatinine, and 2.5 mg/mL of cortisol.

3. Results & Discussion

3.1 Testing binding ability of HSA-MIP via QCM

Figure 4 shows the QCM response of both MIP and NIP-coated electrodes when exposing them to 3 mg/mL standard HSA solution. Sensor signals were read after the respective equilibrium frequency shift was attained. This leads to a decreasing frequency by -520 Hz on the MIP side and -140 Hz on the NIP side, corresponding to -380 Hz mass effect. This response strongly confirms that imprinting was indeed successful with imprinted factor of 3.7 between MIP and NIP. Additionally, all bound HSA could be removed by the washing sequence described earlier to regenerate MIP. Hence, this functional monomer ratio was then chosen as HSA-MIP for further experiments.

**Figure 4.** Frequency response of HSA-MIP and NIP exposed to 3 mg/mL HSA solution

3.2 Selectivity of HSA-MIP

The sensor was exposed to each standard solution comprising of HSA, other urinary substances with ten-time higher physical relevant concentrations containing creatinine (0.13 mg/mL) and cortisol (2.5 mg/mL). After loading 3 mg/mL of HSA, MIP coated electrode obviously leads to substantial frequency signal of -520 Hz. While creatinine adding yields a small negative frequency signal on MIP side of -73 Hz. There is no any decreasing frequency from cortisol loading. Figure 5 displays the QCM results of both MIP and NIP in terms of relative effect compared to the respective HSA signal for a range of substances that are expected in the urine: evidently, creatinine leads to 19% of the HSA signal and cortisol to 0%. In term of surface chemistry, HSA surface contains both negatively and positively charged side chains. Therefore, copolymers of AA and VP as functional monomers complement positive and negative charges, respectively, leading to intermolecular interactions with
HSA. In parallel, creatinine and cortisol, which are a nitrogenous organic compound and a steroid compound, respectively, also compose of both positively and negatively charge side chains, leading to slightly frequency signals of QCM. However, both substances have cyclic structures with low molecular weight, which do not properly correspond to the heart-shaped structure of HSA-MIP cavities. Based on all results shown it can be said that HSA-MIP fits both morphological and functional groups of HSA very strongly.

Figure 5. Selectivity of HSA-MIP

4. Conclusion

All results indicate successful imprinting of HSA-MIP, which is indeed fit both morphological and functional variances between the species very well. This step constitutes the first step in development of a HSA-MIP sensor. This is promising further studies to fully evaluate the sensor system for directly albumin assessment in urine.

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References