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Electrosynthesized molecularly imprinted polyscopoletin nanofilms for human serum albumin detection

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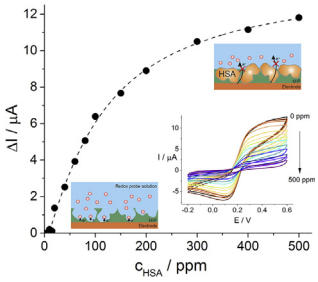
HIGHLIGHTS

- Electrosynthesized polyscopoletin MIP nanofilm is developed for HSA recognition.
- MIP nanofilms were successfully used for label-free urinary HSA assay.
- MIPs show promise for selective detection of high abundance proteins in practice.

GRAPHICAL ABSTRACT



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ABSTRACT

Molecularly imprinted polymers (MIPs) rendered selective solely by the imprinting with protein templates lacking of distinctive properties to facilitate strong target-MIP interaction are likely to exhibit medium to low template binding affinities. While this prohibits the use of such MIPs for applications requiring the assessment of very low template concentrations, their implementation for the quantification of high-abundance proteins seems to have a clear niche in the analytical practice. We investigated this opportunity by developing a polyscopoletin-based MIP nanofilm for the electrochemical determination of elevated human serum albumin (HSA) in urine. As reference for a low abundance protein ferritin-MIPs were also prepared by the same procedure. Under optimal conditions, the imprinted sensors gave a linear response to HSA in the concentration range of 20–100 mg/dm^3, and to ferritin in the range of 120–360 mg/dm^3. While as expected the obtained limit of detection was not sufficient to determine endogenous ferritin in plasma, the HSA-sensor was successfully employed to analyse urine samples of patients with albuminuria. The results suggest that MIP-based sensors may be applicable for quantifying high abundance proteins in a clinical setting.

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1. Introduction

Since the fragility of antibodies is limiting in many biosensing applications their replacement with more robust selective

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receptors received considerable attention [1]. In this respect general synthetic processes with broad applicability in terms of targets to be recognized such as molecular imprinting of polymers are especially appealing [2,3]. Molecular imprinting uses the target as a template during polymerization of functional monomers. Subsequent removal of the template leads to recognition sites in the molecularly imprinted polymer (MIP) that can selectively rebind the target. Extending this principle to macromolecules such as proteins necessitated the implementation of a variety of enabling technologies (e.g., epitope imprinting [4,5], surface imprinting [6–10]) to cope both with the fragility of the target [11] as well as with its limited diffusivity in the cross-linked polymeric matrices. The electrochemical polymerization in this respect offers clear advantages such as performing the polymerization in aqueous conditions that is compatible with the proteinaceous target. Moreover, in terms of chemical sensor fabrication it enables the controlled deposition of MIP nanofilms directly onto an electrode surface [12–14]. While the molecular imprinting concept is fairly universal the functional monomers to provide selective recognition are not, i.e., the library of monomers is rather limited and monomers adequate for a certain protein target may not be necessarily optimal for another [1]. While high affinity MIPs were reported for a number of targets [15,16], the selectivity of protein-MIPs is often enhanced by incorporating in the MIPs compounds known to interact with the target, e.g. substrates or inhibitors of an enzyme target [17,18], aptamers [19] and various nanomaterials [15,20]. Alternatively, rational design of monomers tuned for the specific target and semi-covalent imprinting was also shown to give MIPs with high affinity towards protein templates [21].

However, target specific tuning of the molecular imprinting process departs from the basic universal concept, i.e., to obtain a polymer rendered selective towards different proteins solely by the molecular imprinting process. The difficulty to achieve this goal is largely due to the lack of universally applicable monomer library, i.e., in case of electropolymerized MIPs generally only a single monomer is used for the synthesis of MIPs [12]. Given the diversity of proteins in terms of physical chemical properties it is unlikely to have the full range of interactions for high affinity binding covered by these simple MIPs. Therefore, the success rate of this probabilistic approach was increased in many cases by focusing on proteins with distinctive properties [8,22] that facilitates the likelihood of obtaining binding sites with both strong and selective interaction with the target. However, for “random” protein targets lacking such properties the resulting MIPs rendered selective solely by imprinting are likely to exhibit low to medium affinities, which prohibits their use for practical application where low or even trace amounts of proteins need to be detected. Still their implementation for the recognition of high-abundance proteins seems to have clear niche in the analytical practice. Here we investigated this opportunity through the electrochemical determination of elevated human serum albumin (HSA) in urine by HSA-imprinted MIP sensor. As a reference for a low abundance protein we used ferritin (normal levels are 12–300 ng/mL in the blood). In comparison up to 25 mg/L HSA in urine is considered normal and this value may increase orders of magnitude in case of kidney damage, e.g. elevated urinary excretion of albumin as in microalbuminuria [23] is an early indicator of kidney damage. Studies suggest that microalbuminuria defines a group at high risk of increased cardiovascular morbidity and mortality among patients with diabetes [24–26] or essential hypertension [27]. Moreover, microalbuminuria is associated with increased cardiovascular morbidity even in the non-diabetic, non-hypertensive population [28], pointing out the necessity for routine screening of urinary albumin to enable prediction and prevention of future renal and cardiovascular diseases. There are many dye-binding procedures for rapid screening of elevated HSA [29] that are generally based on the interaction of the albumin and an anionic dye such as bromocresol purple [30]. Since the albumin bound dye has a different absorption maximum than the free dye the HSA can be detected by simple means. However, for very specific HSA determinations clinical laboratories generally use immunoassays, e.g. immunoturbidimetric assays, or separation based methods [31]. While most separation-based methods do not cope with the requirements for high-throughput routine HSA measurements, conventional immunoassays may underestimate the urinary albumin concentration as intact albumin in urine may exist in both immunoreactive and unreactive forms [32]. Therefore, we were interested to explore the use of MIPs, that are expected to give a broader specificity than antibodies, for the quantitation of HSA in urine samples of patients showing microalbuminuria. While several electropolymerizable monomers emerged for protein imprinting applications [12] for preparing the HSA-imprinted MIP we used scopoletin as monomer, which has been introduced by Gajovich-Eichelmann [33] for the electrosynthesis of protein-MIPs and proved to enable the recognition of several protein targets [33–37]. By electropolymerization scopoletin forms an insulating polymer film, the thickness of which can be tuned to match the characteristic dimensions of the protein. The protein binding to the MIP was detected by measuring the oxidative current of a redox probe on the underlying electrode, i.e., the protein binding hinders the permeability of the redox probe through the MIP nanofilm (see Scheme 1).

2. Experimental

2.1. Chemicals and reagents

Scopoletin, human serum albumin (HSA) (isoelectric point (pI) 4.7), ferritin (pI 4.5), avidin (pI 10), lysozyme (pI 11.35) and sodium dodecyl sulphate (SDS) were obtained from Sigma (Steinheim, Germany). Potassium hexacyanoferrate(II) trihydrate ($K_4Fe(CN)_6 \cdot 3H_2O$), sodium hydroxide and Tween 20 were purchased from Fluka (Buchs, Switzerland). All other chemicals used were analytical reagent grade and were used without further purification. Deionized water (DI) of 18.2 MΩ × cm resistivity prepared by a Millipore Milli-Q system was used in all experiments. Phosphate buffered saline (10 mM, pH 7.4, 137 mM NaCl, 2.7 mM KCl) was prepared according to the manufacturer's instructions by dissolution of one phosphate buffered saline tablet (Sigma, Steinheim, Germany) in 200 mL DI water.

2.2. Instrumentation

Electrochemical measurements were carried out with an Autolab potentiostat/galvanostat (model PGSTAT 12, Metrohm Autolab B.V, Utrecht, The Netherlands) controlled by a GPES 4.9 software package (Metrohm Autolab B.V, Utrecht, The Netherlands). A standard three electrode system was used for all electrochemical experiments: a gold disk electrode (2 mm diameter) as working electrode, a Ag/AgCl/KCl (3.5 mol/dm^3) reference electrode, and a platinum wire as counter electrode. Non-specific adsorption and rebinding capacity of MIPs and NIPs were evaluated with a quartz crystal microbalance (eQCM 10 M™ with Reference 600 potentiostat, Gamry Instruments Inc., Warminster, USA). 10 MHz Au-coated quartz crystals (Gamry Instruments Inc., Warminster, USA) were used to prepare MIPs and NIPs for AFM and QCM measurements.

2.3. Electrosynthesis of polymer films

Molecularly imprinted polymers (MIPs) and non-imprinted polymers (NIPs) were prepared by electropolymerization of

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