MOLECULAR IMPRINTING TECHNOLOGY FOR TARGETED ANALYSIS OF PROTEINS

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Abstract: Molecular imprinting has appeared to be an effective technique for creating of selective recognition sites in synthetic polymers. This procedure comprises polymerization of monomer in a presence of target molecules (template). The subsequent template removal forms tailor-made cavities that are complementary in shape and size to the template molecules. For protein imprinting, the choice of the suitable polymers is limited and polymerization conditions need to be optimized. In our work, dopamine monomer was chosen for polymer formation due to its nontoxicity, ease of preparation and self-assembly. For the optimization of conditions, lysozyme with molecular weight of 14.3 kDa was used and the functionality was evaluated by fluorimetry. Different concentration of dopamine and lysozyme for polymerization were tested. Under the optimized conditions, the limit of detection for lysozyme was found to be 7.8 μg/ml. Moreover, conditions for polymer formation for a purpose to reduce the overall time of analysis were investigated. The use of dopamine as a monomer in molecular imprinting shown to be beneficial in many aspects.

Key Words: polydopamine, molecularly imprinted polymer, lysozyme

INTRODUCTION

A molecularly imprinted polymer (MIP) is a polymer with selective recognition sites (Mosbach 1994). In the procedure, a template molecule is added into a solution of suitable functional monomers (Bergmann and Peppas 2008). The most common methods of imprinting are bulk imprinting (for small template) and surface imprinting (cells or viruses); other methods are used as an alternative imprinting strategies, e.g. substructure imprinting, substructural analogues, antibody replica, or molding (Schirhagl 2014). A substantial step is template removal, which is especially challenging when imprinting macromolecules. Template can be removed by using various solvents, such as acids or bases, detergents; the polymer can be heated, or digestive enzymes (proteases) could be used. After removing of the imprinted molecule, the cavities formed in the polymer are complementary to the template in size, shape, and orientation of functionalities are left behind, and are capable to selectively recognize the target molecule (Dechtrirat et al. 2012). The optimization of the polymer structure is extremely important. The polymer should have the following properties: stiffness of the polymer structure, high flexibility, good accessibility, mechanical stability and thermal stability (Wulff 1995).

Molecular recognition is a key principle in biology and bioanalysis (Dechtrirat et al. 2012). The first report about molecular imprinting for detection of protein was published in 1985, when organic silane was used as monomer for polymerization on silica beads and enzyme was entrapped (Glad et al. 1985). The following years were addressed to molecular imprinting of proteins due to the fact that proteins could not be always compatible with organic solvents used during polymer preparation (Bossi et al. 2007). Further, proteins easily subject to external influences, e.g. temperature. In the course of molecular polymerization, it is important to think of functional groups that are able to interact with functional monomer.

It is anticipated that MIPs with specificity for proteins will be applied in medicine, diagnostics, proteomics, environmental analysis, sensors and drug delivery (Bossi et al. 2007). Recently, the MIPs have been widely used for extraction, drug delivery, sensors, catalysis, and drug discovery applications based on their high selectivity, stability and adsorption capacity (Yin et al. 2016). Today, laboratory practice is almost dependent on systems utilizing antibodies for specific protein capture in various assays, for isolation, extraction and biosensors (Turner et al. 2006). The disadvantage is that these systems are often expensive and usually suitable only for single use. There is a growing demand for inexpensive, robust and reusable systems that have the desired level of selectivity and specificity.

Recently, the attention has been focused on the dopamine monomer. Its advantage is that it can self-polymerize in an alkaline or oxidative aqueous solution without a cross-linking or initiating agent (Yin et al. 2015). By using dopamine as a monomer, the time of analysis can be reduced due to facile polymer preparation (Yin et al. 2016). The low cost and limitations in the use of chemicals are also beneficial. As an example, Nematollahzadeh et al. 2013 selectively adsorbed human serum albumin on imprinted polydopamine nanolayer on the surface of porous silica particles. Besides, Lin et al. 2013 prepared a boronate-functionalized imprinted monolithic column with polydopamine coating for glycoprotein enrichment. In other study, proteins were imprinted on the surface of amino-modified Fe_3O_4 nanoparticles using dopamine as a monomer (Gao et al. 2014).

The aim of this work was the optimization of polymerization conditions for dopamine monomer. Different concentrations of monomer, template and polymerization conditions were assessed. The detection limit of fluorescence spectrometric detection of a model protein was also determined.

MATERIAL AND METHODS

Chemicals

All chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA). 20 mM Tris-HCl buffer used throughout this experiment was prepared from Trizma® (TRIS base), pH was adjusted to 8.5 with hydrochloric acid (reagent grade, 35%). The pH was measured by using pH meter WTW inoLab (Weilheim, Germany).

Preparation of polydopamine MIP

Molecularly imprinted polymer (MIP) was prepared by self-polymerization according to literature (Gao et al. 2014, Zhang et al. 2012). In brief, a dopamine hydrochloride (monomer) in different concentrations was dissolved in 20 mM Tris-HCl buffer (pH 8.5). Then, the template molecules of lysozyme were mixed with the monomer in a 1 : 1 (v/v) ratio. 50 µl of the polymerization mixture was pipetted into 96-well microplate (Corning, NY, USA) in 6 repetitions. The resultant polymer was then washed 5 times with mixture of 5% acetic acid (HAc) (v/v) and 10% sodium dodecyl sulfate (SDS) (v/w) to remove the imprinted molecules and once with water. Subsequently, sample (lysozyme dissolved in Tris-HCl buffer) was applied for 1 hour; the microplate was shaken on Eppendorf Thermomixer R W/1.5ml Thermoblock (Eppendorf, Hamburg, Germany). The sample was then removed, unbound target molecules, and interferents were washed out with water. The MIP formation process is shown in Figure 1.

Non-imprinted polymer (NIP), serving as a control, was prepared and treated under the same conditions but with absence of template molecules.

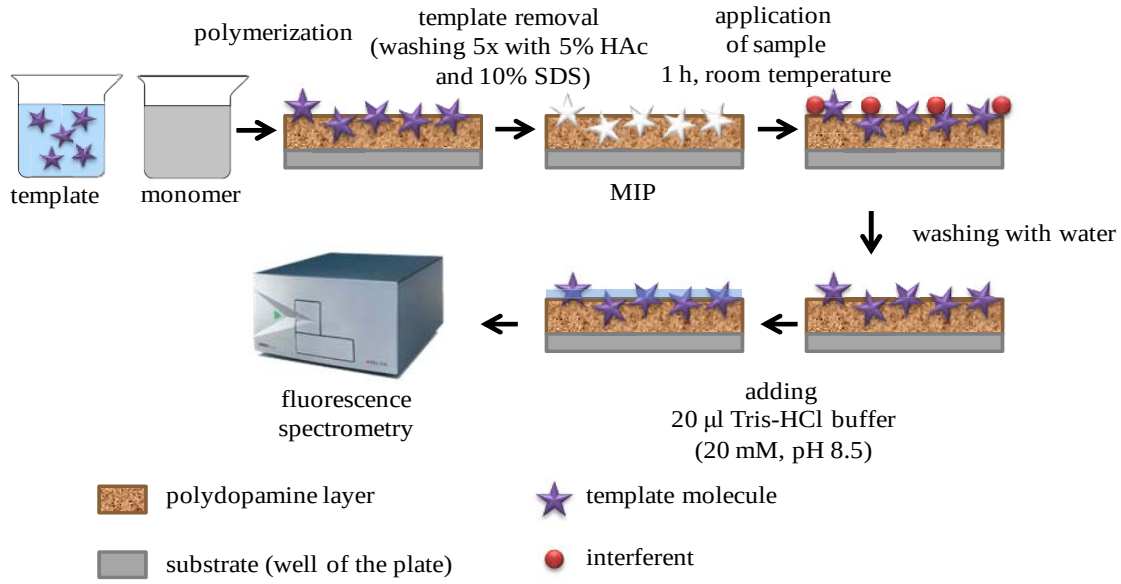
Fluorimetric detection

Fluorescence intensity was measured using fluorimeter Infinite M200 Microplate reader (Tecan, Switzerland). Lysozyme emission (at pH 8.5) was measured at wavelength λ_{ex} 280 nm and λ_{em} 330 nm with gain of the detector set to 100. Before measurements, 20 µl of Tris-HCl buffer was added to the each well.

Statistical analysis

All data were statistically analyzed using Dean-Dixon test (also Q test) and the remote outliers were rejected (with 6 observations at 90% confidence, $Q_{90\%} = 0.56$).

Figure 1 Scheme of MIP polymerization



RESULTS AND DISCUSSION

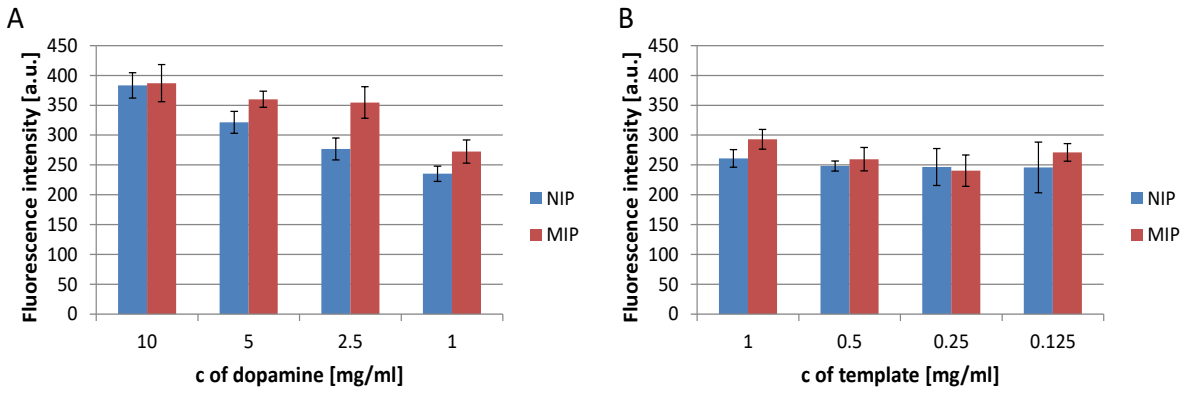
Optimization of monomer concentration

For polymerization, concentrations of dopamine monomer 10, 5, 2.5 and 1 mg/ml were tested. Template concentration of 1 mg/ml was used and concentration of sample was set to 0.125 mg/ml throughout this optimization. Figure 2 A shows fluorescence intensity for different amount of dopamine. Qualitatively, the polymers were evaluated as a relative difference between MIP and NIP. According to our experiment, the best result was achieved in the third case, thus 2.5 mg/ml of dopamine was resulting concentration used further in our experiments.

Optimization of template concentration

In Figure 2 B, the concentrations of template are compared. The initial concentration of 1 mg/ml was sequentially reduced by half. Dopamine concentration was 2.5 mg/ml and sample was 0.125 mg/ml during this experiment. As the most effective, it seems using of template concentration 1 mg/ml (maximal used), where is an evident difference between NIP and MIP, and more template-selective cavities were formed. Other concentrations did not prove to be suitable for the imprinting technique because the fluorescence intensity is comparable between NIP and MIP.

Figure 2 Determination of optimal monomer concentration (A) and template concentration (B)

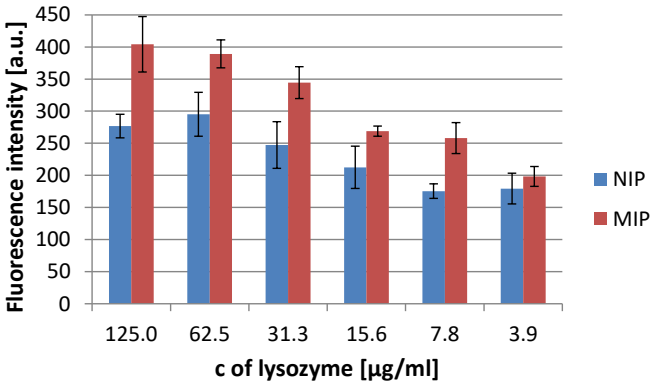


Determination of detection limit

Subsequently, a limit of detection of the created imprinted polymer with optimized concentration of monomer 2.5 mg/ml and template 1 mg/ml was determined. We have examined

concentrations of sample in range from 125.0 to 3.9 µg/ml. Figure 3 shows differences between NIP and MIP among these concentrations. With lower concentration of applied sample, fluorescence intensity declines. For each concentration, there is an obvious difference between NIP and MIP. However, for concentration of 3.9 µg/ml we cannot definitely determine NIP from MIP due to the fact that the error bars overlap. Therefore, the lowest value that we are able to detect using fluorimeter Infinite M200 Microplate reader is 7.8 µg/ml.

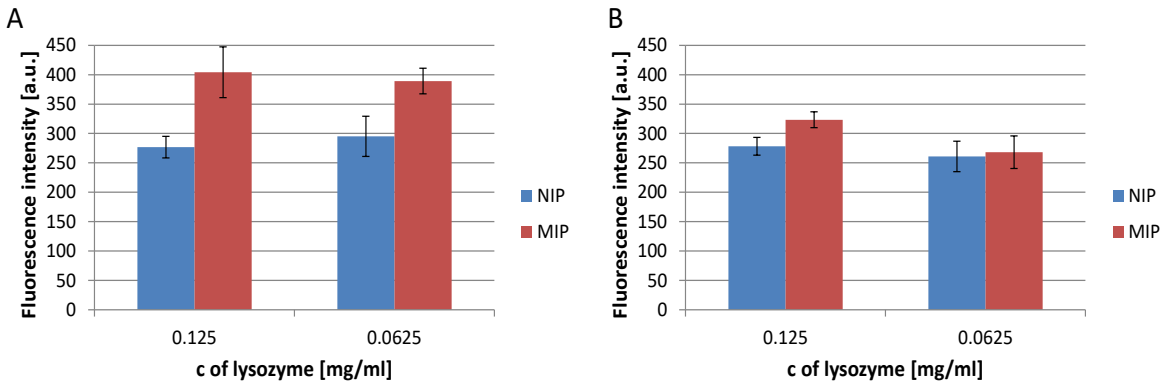
Figure 3 Limit of detection for lysozyme determined using fluorimetry



Optimization of polymerization conditions

The advantage of dopamine polymer is self-polymerization and shorter time of polymerization against most of the polymers. As proposed elsewhere, the polymerization time can be reduced with use of different techniques, such as UV light (Du et al. 2014) or increased temperature (Zhou et al. 2014). We tried to shorten the overall time of the analysis by polymerization of imprinted polymers in a laboratory oven Memmert UE 400 (Schwabach, Germany). NIP and MIP were prepared as mentioned above. We have tested the polymerization at 40°C for 4 hours and compared to our results when the polymer was dried overnight. In both cases, samples with concentration of lysozyme 0.125 mg/ml and 0.0625 mg/ml were applied. As shown in Figure 4 A, we can see the difference between NIP and MIP in both concentrations when the polymer was dried overnight. Nevertheless, in Figure 4 B, there is difference between NIP and MIP only in concentration of applied lysozyme 0.125 mg/ml. This result indicates that the polymerization at the elevated temperature is effective but yields to decreased sensitivity. However, the conditions may be further investigated for dopamine polymerization, more preferably with lower temperature and longer time of drying.

Figure 4 Comparison of dopamine dried overnight (A) and at temperature 40°C for 4 hours (B)



CONCLUSION

In this work were optimized conditions of polymerization dopamine monomer. The best results were achieved with monomer concentration of 2.5 mg/ml; optimal concentration of template (lysozyme) was 1 mg/ml. Under these conditions, fluorimetric method was used for determination of lysozyme due to its simplicity, low costs and effectivity. We were able to measure the protein

in concentration as low as 7.8 μg/ml. To accelerate the dopamine polymerization, conditions 40°C for 4 hours were tested. Under these conditions, the polymerization was effective; however the created polymers did not attain such functionality as when they were dried at room temperature overnight.

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