CHEMICAL SG-SELEX ON THE NONPOROUS SILICON SUBSTRATE CAN GENERATE HIGH AFFINITY ssDNA APTAMERS AGAINST NON-SOLUBLE CHEMICALS

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ABSTRACT

We have described the development of Sol-gel mediated-Systematic Evolution of Ligands by Exponential Enrichment (referred to as “SG-SELEX”) technique. This method allowed us to isolate aptamers against chemicals with non-soluble agrochemicals, azoxystrobin. The porous silicon surface was also modified for improving the adhesiveness of sol-gel microdroplets. Through 5 rounds of selection, the high-affinity ssDNA aptamers were generated, and selected ssDNA aptamer, Azo 5-3, Azo 5-6 and Azo 5-29. Our data demonstrate that the sol-gel is a convenient partitioning and simplified retrieval method in SELEX, and isolated aptamer hold great promise for capturing chemicals as a high sensitive biosensing material.

KEYWORDS: SG-SELEX, chemical immobilization, azoxystrobin, sol-gel microarray, porous silicon surface, aptamer

INTRODUCTION

In the conventional SELEX process capturing tags/ligands is a requisite for immobilizing target chemicals to supporting matrix, and so, the danger of selecting aptamer species which bind to the coupling matrix and target cooperatively is always present[1]. In SG-SELEX, active target chemicals were immobilized on sol-gels without any ligand/linkage/coupling agent [2]. Moreover, binding compounds can enter inside the sol-gels and interact with target molecules in the high surface area nanostructured sol-gel mineral network. The porous silicon surface was also modified in this study for improving the adhesiveness of sol-gel microdroplets on the substrate. This is a key step, enabling high throughput screening for binding partners in aptamer libraries. Previous work on aptamer selection for low molecular chemicals has displayed major problems in the detachment of sol gel spots from regular substrates during the SELEX sequence. The reason for this is the substrate incompatible solvent systems. Hence automated efforts in aptamer screening against pesticides and pollutants have been hampered.

EXPERIMENTAL

Reagents and materials BPA (4,4’-dihydroxy-2,2-diphenylpropane, Sigma-Aldrich, USA) was dissolved in 50% dimethylformamide (DMF) at a final concentration of 20 mM. Azoxystrobin (Methyl (E)-2-[2-[6-(2-cyanothoxynaphthyridin-4-yloxy]-phenyl]-3-methoxyacrylate) with a purity >99.6% (w/w) was purchased from FLUKA-RIEDEL DE HAE’N (SIGMA–ALDRICH CHIMIE). The stock standard solution (50mM based on an average molecular mass 403.4) was prepared by dissolving with 50% dimethyl sulfoxide (DMSO, Fluka). It was kept away from light in the box and stored at 4°C. And 250uM Azoxystrobin in binding buffer (25 mM Tris-HCl, 100 mM NaCl, 25 mM KCl, 10 mM MgCl2, 5% DMSO, pH 8.0) was finally prepared as a working solution.

Sol-gel preparation We utilized the Sol-gel materials for immobilizing aptamers according to manufacturer’s recommendation (SolB complete kit, PCL Inc., Korea, www.pelchip.com). In detail, using the optimized sol-gel formulations for small molecules (SolB I 26.2%; SolB II 10.5%; SolB III 7%; SolB H 12.5%; SolB S 11%), the 100 μM of chemicals, BPA and Azoxystrobin were mixed with SolB reagents and arrayed with controls (N; negative control and P; reference control with Cy-3 dUTP) using the non-contact microdispensing instrument (DW-SolB, PCL Inc. Korea).

Preparing the porous silicon surface The porous silicon surfaces were obtained by electrochemical dissolution of silicon wafers. The detail descriptions of fabrication step and chemical preparations for silicon etching were written on the reference. In summary of fabrication, we used a two-compartment electrochemical cell with sapphire glass (Melles Griot BV) on one side to allow for illumination during anodizing. Wafers were etched at constant current 2mA/cm² for 10 minutes. The backsides of silicon were illuminated through anodization period using 100-W halogen lamp (Osram, Germany) at a distance of 10 cm from the window on the back of the electrochemical cell. The SEM images of porous silicon surfaces were analyzed using a JEOL JSM-6700F field emission scanning electron microscope (JEOL, Japan). Before SEM analysis, a 10-nm thick of Platinum was deposited on the samples.

RESULTS AND DISCUSSION

For the selection of high affinity aptamers against chemicals, a wide variety of molecular immobilization techniques have been explored as means to improve the selection efficiency, including the reduction of lengthy screening process. Sol-gel was applied to immobilize chemicals and porous Si chip surface was introduced here as a new substrate. Sol-gels can immobilize target chemicals on their networks which consist of complicate nano size channels and pores. It is...
possible that binding probes like aptamers can enter and exit through the channel freely. Also, comparing the protein immobilization to the porous substrate by the physical adsorption, free aptamers were not reactive to the porous surface. Since this lead to the decrease of background levels and nonspecific binding, it can be an additional beneficial property of porous silicon surface in an aptamer application.

Figure 1. FESEM images of sol-gel and porous silicon surface

Figure 1 show sol-gels fixed on the porous silicon substrate. The diameter of sol-gel drop is ~ 250 µm and height is < 20µm. Sol-gel pores were observed in 140 nm~1 µm dimensions which is sufficient for the access of binding probes. As a proof of concept, we utilized the model chemical-aptamer pair, bisphenol A (BPA) and the specific aptamer (apt-#3). BPA containing sol-gels were arrayed on the porous surface with 50 nl/spot. Fluorescent labeled anti-BPA aptamer #3 was incubated with sol-gels and the resultant assay chip was analyzed to figure out the capturing ability of aptamers against chemicals in sol-gels. To investigate the specific interaction of aptamers on the porous chip, BPA containing sol-gels were spotted along the alphabets ‘L, U, N, D’ sequentially and negative sol-gel microdroplets (without BPA) were filled up in the 8 x 8 sol-gel array. Cy3 labeled aptamers were incubated in each assay well and the resultant chips were scanned. Since target bound aptamers was completely released by heat, the retrieved aptamers can be applied to the consecutive SELEX rounds.

Figure 2. Specific interaction between chemical and aptamer: BPA contained sol-gels were spotted along the alphabets ‘L, U, N, D’ sequentially and negative sol-gel microdroplets (without BPA) were filled up in 8 x 8 sol-gel patterned array. Cy3 labeled BPA aptamers were incubated in each assay well and then, resultant chips were scanned.

Based on this strategy, we isolated aptamers against chemicals with low solubility, azoxystrobin, a fungicide used in agriculture. Azoxystrobin possesses the broadest spectrum of activity of all presently known antifungals and has water pollutant potential. The aptamer pools eluted from the 4th and 5th round were cloned and individual clones were sequenced. Table 1 shows homologous aptamer pairs classified and placed in the column of eight sections based on the primary sequence and homology percentages.
CONCLUSION

Our proposed chip assembly offers a promise of a convenient method to isolate target specific aptamers and design sensitive microarray chips for monitoring residual agricultural chemicals exceeding the acceptable limit. Also, high affinity aptamers can be applied to a filtrate plant for a removal of pesticides residue from contaminant water sample.

ACKNOWLEDGEMENTS

This study was supported by the the Korea Research Foundation Grant (KRF-2008-532-D00003/2009-535-D00004).

REFERENCES


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