

Screening and Isolation of DNA aptamers against Agrochemicals by using PS-SELEX chip

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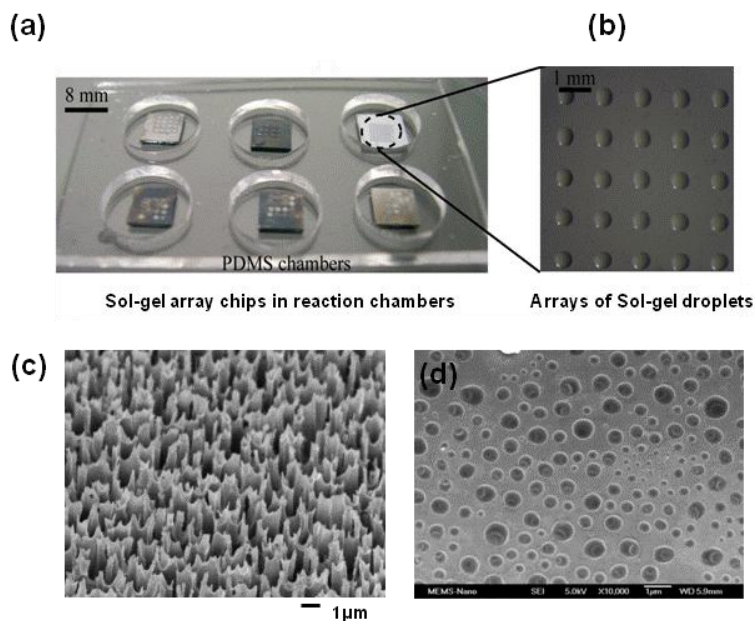
Abstract

We have described the development of **Porous Substrate mediated-Systematic Evolution of Ligands by Exponential Enrichment** (referred to as “PS-SELEX”) technique. This method allowed us to screen and isolate high specific aptamers against chemical compounds. Azoxystrobin as a target chemical is a fungicide commonly used in agriculture and has water pollution potential. This is generally used as an active agent protecting plants and fruit/vegetables from fungicidal diseases (1). In PS-SELEX, azoxystrobin was immobilized on sol-gel networks. Especially, sol-gel immobilization is not necessary for linkage/coupling agent (2). Moreover, interacting binding materials can enter into along the complicate internal channels of sol-gels and release to outside. For improving an adhesiveness of sol-gel microdroplets on the substrate, the porous silicon substrate was newly modified in this study (Figure 1). The aptamer pools eluted from the 5th selection rounds were cloned and individual clones were sequenced. Identical DNA aptamer pairs were classified and we finally choose the two aptamer species, Azo 5-3 and Azo 5-6 (Table 1), and analysis of the secondary structure of the isolated aptamers was performed with a free energy minimization algorithm using the *Mfold* program (3). In contrast to traditional chemical SELEX, our strategy provides the following advantages: Simple immobilization of chemical compounds, Easy to handle of aptamers in SELEX process, Decrease the non-specific bind to chip surface. Our data demonstrate that the sol-gel is a convenient partitioning and simplified retrieval method in PS-SELEX process, and isolated aptamer hold great promise for capturing pesticides as a high sensitive biosensing probe.

References

- [1] Ji-Young Ahn, *Analytical Chemistry*, **84** (2012) 2647-2653
- [2] Ji-Young Ahn, *Oligonucleotides*, **21** (2011) 93-100
- [3] <http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/dna-form1.cgi>

Figure 1.



PS-SELEX was performed on the small dice of porous silicon substrate. Azoxystrobin contained sol-gels were spotted on the porous chip surface. Considering the 10 to 1 ratio between the number of random ssDNA pools and target chemicals, totally 12 pmole of azoxystrobin were participated in a single round. After stable gelation of sol-gels, 120 pmole of random ssDNA pools were applied to sol-gel integrated chip. Aptamer binders were collected by heat, amplified and regenerated to ssDNA for next round SELEX. (a) assay chamber, (b) sol-gel droplets, (c) SEM image of the PS chip surface, (d) SEM image of sol-gels.

Table 1. Sequence of isolated DNA aptamers

Identity	Sequence	Homology
Azo4-18	-GCCAATCGGCCAAGTCCTGTCTATGCAGCCTGCATCCCT-	
Azo4-9	GTTTCGATCGGGTTAATGCT-CCTATGAAGGTGCCAACGCTG	55%
	* * * * *	
Azo4-31	CTAAGTAGGGGAC-GTCGGACATCACC--TTTCAAATTACCC	
Azo4-23	CGAATCATCGATTGTTCGTTCTCTTCCGTTTCAAATTAC--	55%
	* * * * *	
Azo4-18	-GCCAATCGGCCAAGTCCTGTCTATGAAGCCTGCATCCCT-	
Azo4-9	GTTTCGATCGGGTTAATGCT-CCTATGAAGGTGCCAACGCTG	58%
	* * * * *	
Azo4-11	TGTTATGATGCACTAGCACATCACACGAC-ACGAGCTAATG	
Azo4-24	-ATAATGCGATATTAGCTCATGGGATCACCACGAGCATGTG	58%
	* * * * *	
Azo4-8	-----GGCCAATCTGTCAATTGCGTCGCGAGTCGAAGGTGAGGGGG	
Azo4-1	TCCAAGGCCA--CTGTCATTGCGTCCCGAGTCGAAGGTGAGG---	85%
	* * * * *	
Azo5-30	TCGGTTAGGGGGCTTCGGTTAGGGGGCTCAATCTAATCGA	
Azo5-38	TCGGTTAGGGGGCTTAG---AAGCCGCGCGGTGTAGCCTGCGG	63%
	* * * * *	
Azo5-3	GGCTTTATTTTCGCCCACACGCAGCTTTTGTAACGGGTCGC-	
Azo5-29	GCTTTATTTTCGCCCACACGCAGCTTTTGTAACGGGTCGCT	98%
	* * * * *	
Azo5-6	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	
Azo5-7	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	
Azo5-33	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	100%
Azo5-34	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	
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