(Two pages abstract format: including figures and references. Please follow the model below.)

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

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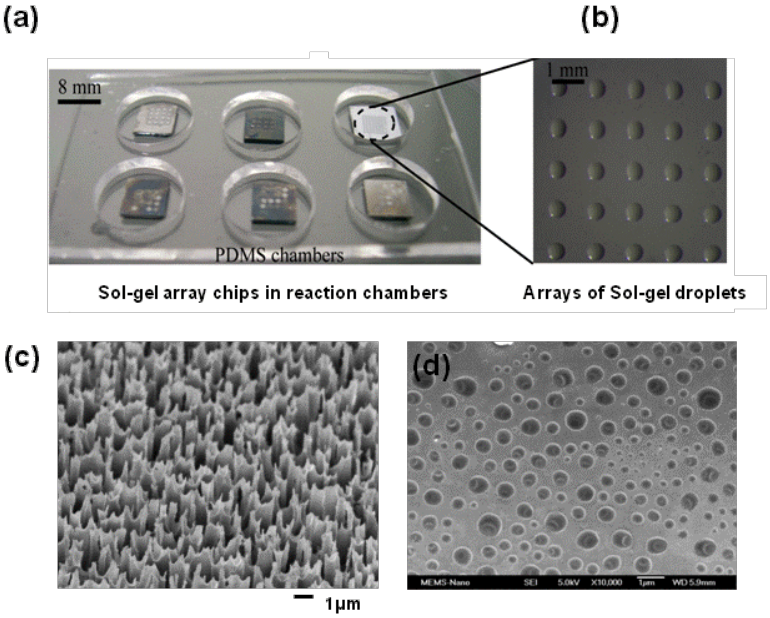
Abstract

We have described the development of **P**orous **S**ubstrate mediated-**S**ystematic **E**volution of **L**igands by **Ex**ponential 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 5^{th} 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	GTTCGATCGGGTTAATGCT-CCTATGAAGGTGCCAACGCTG	55%
	* ***** * * ** ***** ** ** * **	
Azo4-31	CTAAGTAGGGGAC-GTCGGACATCACC--TTTCAAATTACCC	
Azo4-23	CGAATCATCGATTTGTTCGTTCTCTTCCGTTTCAAATTAC--	55%
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Azo4-18	-GCCAATCGGCCAAGTCCTGTCTATGAAGCCTGCATCCCT-	
Azo4-9	GTTCGATCGGGTTAATGCT-CCTATGAAGGTGCCAACGCTG	58%
	* ***** * * ** ******** ** * **	
Azo4-11	TGTTATGATGCACTAGCACATCACACGAC-ACGAGCTAATG	
Azo4-24	-ATAATGCGATATTAGCTCATGGGATCACCACGAGCATGTG	58%
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Azo4-8	-----GGCCAATCTGTCATTGCGTCGCGAGTCGAAGGTGAGGGGG	
Azo4-1	TCCAAGGCCA--CTGTCATTGCGTCCCGAGTCGAAGGTGAGG---	85%
	***** ********** **********	
Azo5-30	TCGGTTAGGGGGCTTCGGTTAGGGGGCTCAATCTAATCGA	
Azo5-38	TCGGTTAGGGGGCTTAG---AAGCCGCGCGGTGTAGCCTGCGG	63%
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Azo5-3	GGCTTTATTTCGCCCACACGCAGCTTTTGTAACGGGTCGC-	
Azo5-29	GCTTTATTTCGCCCACACGCAGCTTTTGTAACGGGTCGCT	98%
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Azo5-6	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	
Azo5-7	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	
Azo5-33	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	100%
Azo5-34	TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG	
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