SEPARATION OF ssDNA-LYSOZYME COMPLEXES WITH HIGH AFFINITY AND SPECIFICITY BY HIDROPHOBIC ADSORPTION

Rubén Medina Flores, Juan Carlos García Solís, Edgar Salgado Manjarrez, Juan S. Aranda Barradas
Unidad Profesional Interdisciplinaria de Biotecnología IPN
Av. Acueducto s/n Col. Barrio La Laguna Ticoman Del. Gustavo A. Madero C. P. 07340 México D.F
email: Esalgado@gmail.com

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Introduction. Aptamers are synthetic, single-stranded DNA or RNA molecules that fold up into unique 3-D structures, allowing them to bind specifically to other target molecules(1). DNA or RNA aptamers can be routinely isolated from synthetic combinatorial nucleic acid libraries by *in vitro* selection, known as systematic evolution of ligands by exponential enrichment (SELEX)(2). The separation of target bound and unbound oligonucleotides during the process is the crucial step for successful aptamer selection (3). A conventional separation method is affinity chromatography (4), filtration using nitrocellulose filters (5) or magnetic beads for target immobilization low amounts of target. This work describes a new method to separate ssDNA-Lysozyme complexes.

Methods. The starting aptamer library consisted of a 80-mer in which two defined primer binding sites flanked a central region comprising 40 random nucleotides AGCAGCACAGAGGTCAGATG-N_{30}-GGCTACCTTGTTACGACTTG. Forward and reverse primers were synthesized to enable PCR amplification of oligonucleotide library (Fwd: AGCAGCACAGAGGTCAGATG and Rev: CAAGTCGTAACAAGGTAGCC). In Fig 1. Can be seen experimental method utilized.

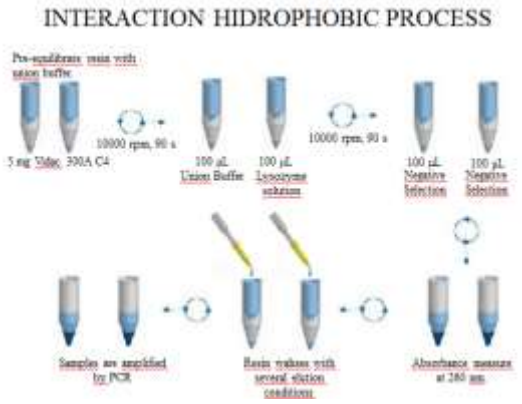

Figure 1. The separation method of target bound and unbound oligonucleotides to obtain oligonucleotides with high affinity and specificity toward lysozyme.

Results.

Control experiment Positive selection

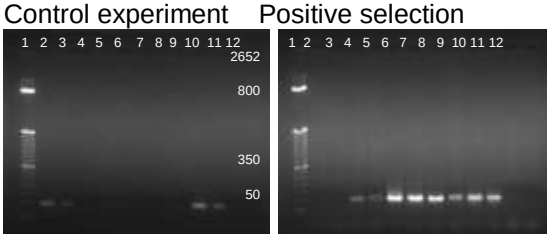

Figure 2. Samples obtain to different elution conditions to separate ssDNA bound to lysozyme. (1) Positive PCR control, (2) sample after bind ssDNA confront lysozyme. (3-7) wash buffer Tris-HCl 10 mM pH 7.5 100 mM NaCl. (8) Elution buffer Tris-HCl 10 mM pH 7.5 0 mM NaCl. (9-11) Elution with acetonitrile to 10, 20, 30 %. (12) Negative PCR control.

We selected Vydac reverse-phase C4 media as hydrophobic support because it presents a weaker hydrophobicity. We expected that DNA-protein complexes would be separated from free linear DNA through a decrease in strength ionic or modified hydrophobic characteristics of the mobile phase through an organic modifier without dissociating the protein from solid.

Conclusions. The reported method is quite simple and cheap for the development oligonucleotides with high affinity and specificity toward lysozyme like substances that can be hydrophobically adsorbed, such as proteins. As the dissociation constant reached micromolar levels in a single selection round, this method is competitive with other methods of separation DNA-protein complexes like capillary electrophoresis or streptavidin magnetic beads.

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References.

1. Proske D, Blank M, Buhmann R, Resch A. (2005). *Appl. Microbiol. Biotech.* 69: 367-374.
2. Wochner A, Cech B, Menger M, Erdmann V, Glökler J. (2007). *Biotechniques*. Vol 43 (3):344-353.
3. Stoltenburg R, Reinemann C, Strehlitz B. (2006). *American Biotech Lab*. Vol 24 (1):18-20.
4. Ellington A and Szostak J. (1990). *Nature*. Vol 346: 818-822.
5. Tuerk C and Gold L. (1990). *Science*. Vol 249: 505-510.