

BEAD-BASED APPROACHES TO DEVELOP THIOAPTAMERS FOR DIAGNOSTICS AND THERAPEUTICS

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ABSTRACT

In this short paper, we will outline our novel, integrated bead-based oligonucleotide selection procedures including the automation of the split-pool synthesis of a one-bead one-thioaptamer library, high-throughput flow cytometric sorting and a PCR-based sequencing. Additionally, we will also discuss one promising thioaptamer that has been shown in preliminary animal therapeutic dosing to increase survival in animal models of infection with West Nile virus. Moreover, we will present our ongoing work on a thioaptamer proteomics chip.

INTRODUCTION

Aptamers (1,2) with normal phosphate ester backbones obtained from the *in vitro* selection process were discovered in the early 1990s and have proven very useful for target validation, drug discovery, diagnostics and therapy (3). In December 2004, an aptamer for vascular endothelial growth factor, Macugen, made headlines as the first of its class to be approved by the US Food and Drug Administration. However, traditional *in vitro* aptamer selection methods have several inherent limitations. First, the selection process is a time consuming process which involves multiple rounds of alternating selection and amplification steps. Second, the polymerases required to PCR amplify the target binding sequences accept only a limited number of substrates and templates. Consequently, only a limited number of aptamer modifications are possible to select for using *in vitro* selection. For this reason, post-selection modification is often required to increase binding affinity and stability. Thioaptamers (3), which are thiophosphate ester backbone modified nucleic acid aptamers, overcome the limitations of normal aptamers. The sulfur backbone modifications offer enhanced nuclease resistance and target affinity relative to normal aptamers. Over the past several years, we have demonstrated proof-of-principle that a novel bead-based approach (4,5) can be employed to develop aptamers, thioaptamer and other modified aptamers as well.

RESULTS AND DISCUSSION

We have previously developed a split-pool synthesis strategy to manually synthesize bead-based nucleic acid combinatorial libraries (4). To expedite thioaptamer library production, a patent-pending device that automates the split synthesis called the Integrated Generator for Oligonucleotide Research (IGOR) was designed and assembled. The four column IGOR was integrated with two Expedite 8909 Systems (Applied Biosystems) and enables generation of a totally random bead-based library of thioaptamers in a timely manner. The monomer added to each column at each step can be a standard phosphoramidite or a thiophosphoramidite. This allows us to synthesize a random sequence thioaptamer library with randomly dispersed phosphate, monothiophosphate or dithiophosphate linkages. Such a library allows us to simultaneously select optimal target binding sequences and backbone modifications in a single

selection round. The invention and reduction to practice of the IGOR significantly shortened the time needed to identify and select thioaptamers for specific targets, removing a significant barrier to widespread thioaptamer application (particularly those modified with dithiophosphates that cannot be identified through normal enzymatic, iterative selection cycles).

Thioaptamers possess binding affinities and specificities similar to antibodies. They are a possible complements to or replacement for currently available antibodies and offer several advantages. First, thioaptamers are chemically synthesized and amenable to a variety of chemical modifications (fluorescent dyes, biotin, etc.) Second, thioaptamers undergo reversible denaturation, so surfaces containing them may be regenerated by a heating and cooling cycle. Last, thioaptamers can be developed for targets that are difficult or impossible to raise antibodies against (i.e. low or non-immunogenic targets, toxins, etc.). We have already demonstrated thioaptamers' potential and versatilities in diagnostic assay formats where they can capture very low abundance of proteins in crude cell extracts (6) and biological toxins. Our recent data underlines the potential of thioaptamers as powerful diagnostic reagents in sophisticated assay formats.

With the Food and Drug Administration approval of the first aptamer drug "Macugen" for the treatment of age-related macular degeneration at the end of 2004, aptamer technology achieved a breakthrough in therapeutic applications. Our nuclease resistant thioaptamer XBY-S2 targeting AP-1 has been tested for anti-viral activity against West Nile virus. Our preliminary therapeutic dosing data has demonstrated increased animal survival rates in our animal model experiments after the animals were infected with West Nile virus (7,8).

CONCLUSIONS

An efficient method for identifying thioaptamers for their targets *via* bead-based thioaptamer selection has been proved in principal. Because of their potential for high affinity, high specificity and high stability, the rapid generation of thioaptamer ligands by bead-based selection methods and the unique options to fully control the experimental setting open up many opportunities for early target validation, diagnostic applications and drug development.

ACKNOWLEDGMENTS

This research was supported by grants to Dr. Xianbin Yang from Mike Hogg Fund and NIH (1R03CA121353-01); to Dr. David Gorenstein from DARPA (P42296LS0000), NIH (U01 A1054827, IP30 ES06676; N01 HV28184; U01 AI 60616), DoD (DADD13-02-C-007 and contract number W911 SR-04-C-0065 with the U.S. Edgewood Chemical Biological Center), the Welch Foundation (H-1296), and the State of Texas Advanced Technology Program (004952-0038-2003).

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