Selection and analytical applications of aptamers

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Advances in systematic evolution of ligands by exponential enrichment (SELEX), a selection protocol for aptamers, have resulted in increased applications of DNA and RNA aptamers in developing analytical techniques. We review recent developments in SELEX techniques as well as new aptamer-based bioanalytical applications.

Keywords: Affinity binding; Aptamer; Bioanalytical assay; Biosensor; Chiral separation; In vitro selection; Protein; SELEX

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1. Introduction

Nucleic acids possess desirable properties for use in analytical applications. Conventionally, they are used as probes that hybridize with and detect complementary target RNA and DNA sequences. In the last decade, short synthetic DNA and RNA sequences known as aptamers have been used as ligands to bind to non-nucleic acid targets with high specificity and affinity. Dissociation constants for typical aptamer binding are in the micromolar to low picomolar range [1,2]. This binding is not through sequence hybridization but through binding of stable, complex stem-loop and internal loop structures formed by the nucleic acids [1].

Aptamers are commonly evolved *in vitro* via a combinatorial chemistry technique known as systematic evolution of ligands by exponential enrichment (SELEX) [3]. They can be selected using a variety of targets from small molecules to whole organisms [4]. The ability to synthesize aptamers for a variety of targets has contributed to a wide range of analyses using aptamers.

Another factor allowing the widespread application of aptamers to bioanalysis is that aptamers offer benefits for analytical applications when used in place of antibodies [5]. These benefits derive mainly from temperature stability and ease of aptamer production.

Many of these analytical applications of aptamers, such as those based on chromatography, mass spectrometry, molecular beacons, and some label-free biosensors, have been reviewed previously [6–9], and will not be discussed further. This review focuses on recent developments in SELEX analytical applications, as well as variations in SELEX techniques and the ramifications of such variations for aptamer characteristics.

2. SELEX techniques

Fig. 1 shows the basic principle of most SELEX procedures [10]. Attempts to improve SELEX have led to the development of different techniques, delineated by variations in starting library, target molecule, and experimental protocol. These variations result in differences in aptamer affinity and specificity as well as selection efficiency.

2.1. Libraries

A typical SELEX library comprises single-stranded RNA or DNA, in which a central region of randomized sequence is flanked on either side by fixed primer sequences for PCR and/or RT-PCR amplification. The first SELEX procedures used randomized single-stranded RNA libraries, in which the random sequence region was 100 nucleotides (nt) in length [1] and 8 nt [3], respectively. Since then, many other SELEX procedures have been carried out using randomized ssRNA or ssDNA libraries, typically containing 10^{13}–10^{15} different randomized sequences [11]. Although any length of randomized region

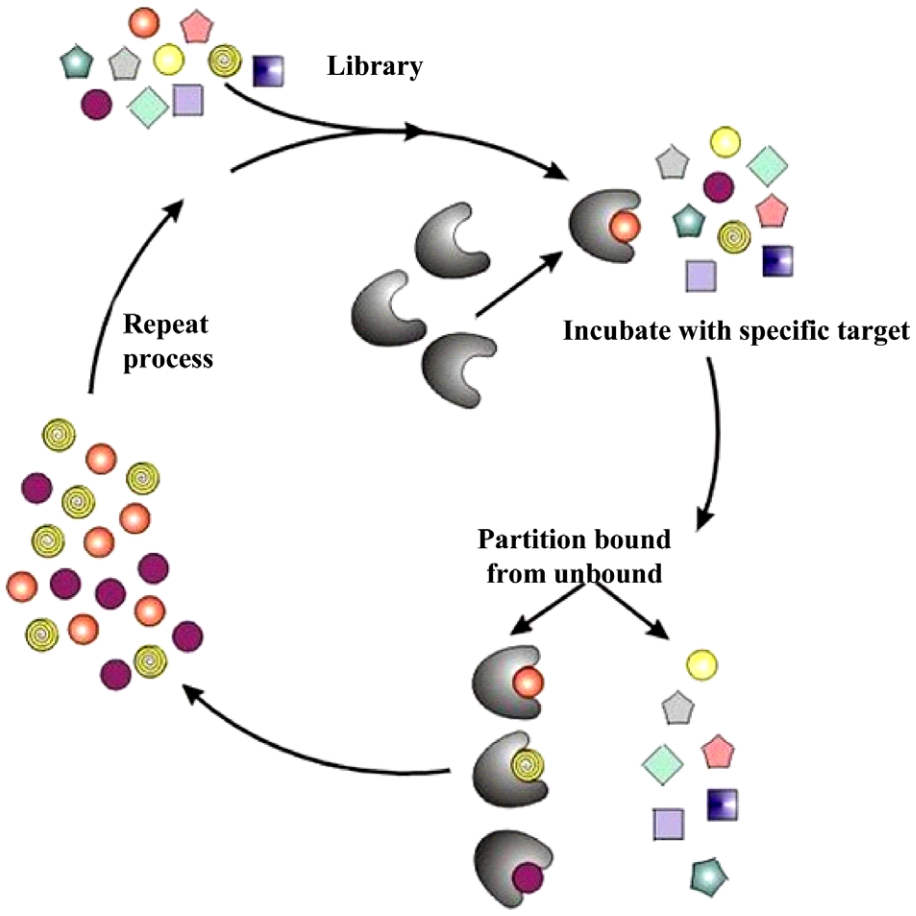

Figure 1. Schematic of Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [10]. Briefly, typical SELEX procedures involve binding a random nucleic-acid library to a target, separating the bound and unbound nucleic acids, and amplifying the bound nucleic acids by the polymerase chain reaction (PCR) for use in the next round of selection [1,3]. After each round of selection, a smaller pool of nucleic acid sequences binding to the target is retained and the unbound nucleic acids are discarded. Typically, 8–15 rounds of SELEX are carried out in order to generate a pool of aptamers with sequences enabling the highest binding affinity for the target. These aptamers can then be cloned and sequenced. (Reprinted, with permission, from the *Annual Review of Medicine*, Volume 56 ©2005 by Annual Reviews www.annualreviews.org)

may be used to provide 4^n theoretical random sequences, in reality, synthetic limitations prevent most libraries from containing more than 10^{16} random sequences (e.g., for a library to contain one molecule each of 10^{16} random sequences, a minimum of 16.7 nmole nucleic acids (or 0.4 mg of nucleic acids containing a 40 nt random region and two 20 nt primer regions, ~25 kDa) is required). In practice, therefore, randomized regions of 30–60 nt are most common [11]. DNA libraries are particularly useful for applications requiring increased aptamer stability, such as biosensing, environmental monitoring, and therapy. However, in many cases, RNA libraries yield aptamers with higher binding affinities than DNA libraries due to the ability of RNA to take on a wider variety of conformations than DNA.

Several SELEX techniques have been developed using modified randomized ssRNA or ssDNA libraries. Photocrosslinking procedures, described in more detail later, use ssDNA libraries substituted with fluorophore-modified nucleotides, such as 5′-BromodeoxyuridineUTP or 5-iodouracil [12,13]. Upon irradiation with a laser, these nucleotides facilitate crosslinking of an aptamer to the target.

Genomic SELEX procedures make use of ssDNA or ssRNA libraries derived from the genome of an organism of interest to probe for genome sequences that interact with a specified target [14–16] Currently, there are *Escherichia coli, Saccharomyces cerevisiae* and human genomic DNA libraries that are able to be transcribed into RNA or amplified by PCR [14]. In all other respects, genomic SELEX is similar to conventional SELEX. Unfortunately, genomic SELEX procedures tend to be biased towards strong or highly abundant interactions and yield many interactions that may not be important *in vivo*. So far, genomic SELEX has yielded aptamers with relatively low binding affinities and high dissociation-constant (K_d) values of 1.9–12.8 μM in 5 rounds of selection [15].