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Review

Nucleic acid aptamers as high affinity ligands in biotechnology and biosensorics

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ABSTRACT

Aptamers are small nucleic acid molecules capable of binding to a wide range of target molecules with high affinity and specificity. They have been developed and widely used not only as research tools, but also as biosensors, specific antagonists, and diagnostic markers and as protein purification platform for many pharmaceutical and clinical applications. Here, in this paper we will explore biochemical aspects of aptamer–target interactions and show why aptamers rival antibodies in target recognition and purification procedures. This review will focus on strategies of using aptamers as affinity ligands for molecules of therapeutic and pharmaceutical interest including applications in chromatography and capillary electrophoresis for protein and small molecule purification. Moreover, we will also discuss aptamers whose binding parameters can be controlled on demand for diagnostic approaches and used as sensitive receptors in biosensorics. Aptamers have opened up exciting fields in basic and applied research of pharmaceutical and biotechnological interest.

Contents

1. Introduction

Aptamers are single stranded DNAs, RNAs or a combination of these with non-natural nucleotides capable of adopting three-dimensional structures which interact precisely and specifically with a target molecule [1]. Dependent on the target functional groups, hydrogen bonds, electrostatic interactions, stacking interactions and hydrophobic effect are the driving forces, which are commonly involved in the molecule binding to the aptamer [2]. The equilibrium dissociation constants (K_d) of aptamer–target complexes are usually in the pico- to micromolar range, and can distinguish enantiomers of small molecules or minor sequence variants of macromolecules with frequently several orders of magnitude K_d ratio [3]. Aptamers were developed as specific ligands of a large number of small molecules, soluble proteins, membrane-bound receptors, cell surface epitopes, entire cells and even whole organisms. Some of these have been also included in clinical trials for studying their therapeutic potential [4,5].

Aptamers are commonly identified from vast combinatorial libraries that comprise 10^{13}–10^{15} of different sequences by a process known as systematic evolution of ligands by exponential enrichments (SELEX) (see for recent reviews [6,7]). In vitro selection

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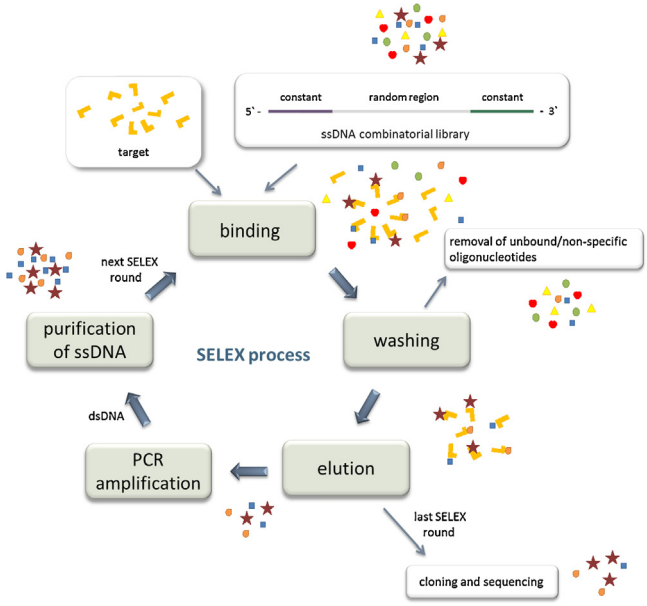

Fig. 1. SELEX. SELEX (Systematic Evolution of Ligands by EXponential enrichment) process is a protocol for the in vitro selection of aptamers (nucleic acids) capable of binding to a specific target with high selectivity and sensitivity because of their three-dimensional shape. Starting point of a typical SELEX process is a chemically synthesized random DNA or RNA oligonucleotide library consisting of about 10^{13}–10^{15} different sequence motifs. The method involves iterative steps of selection and enzymatic amplification driving the random pool toward a population containing of relatively few aptamers with optimized structural motifs.

experiments involve an iterative process of adsorption, partitioning, recovery and amplification of the selected sequences to provide an enriched pool of nucleic acids for the next cycle of selection and amplification (Fig. 1). These cycles are typically repeated until target-binding oligonucleotides have evolved from the population. Typically, after 5–15 cycles of the SELEX process, the library is reduced to contain only a small number of aptamers which exhibit particularly high affinity to a target [8]. Selected nucleic acids are isolated and identified by cloning and DNA sequencing. Aptamers are identified by comparison of conserved sequence motifs in the previous random regions by bioinformatics tools and further characterized by screening for binding and/or biological activity.

Aptamers, also known as "chemical antibodies", have been suggested to be good candidates for substituting antibodies or just being used as their alternatives in diverse pharmaceutical applications and biotechnology as well as in basic research [1,9]. Aptamers present many relevant advantages over antibodies. The SELEX process does not involve a living system and is not depending on a binding reaction in a biological system. The method is based entirely on in vitro selection by which oligonucleotides are screened for their affinities for a target molecule. Selection parameters (temperature, ionic strength, pH etc.) can be adjusted to applications followed for each aptamer. After identification of target-binding sequences, large-scale production of aptamers by chemical synthesis can be performed in fully automated machines with little or no batch-to-batch variation. The greatest advantage of aptamers over protein-based antibodies is their stability at harsh chemical and physical conditions. Aptamers recover their native conformation and bind to targets after re-annealing [10]. For applications in the medical area much effort has been made in using oligonucleotide libraries with chemical modifications of nucleic-acid backbones or libraries which include different functionalities that are present in naturally occurring nucleotides. Modifications aim to increase aptamer potency by enhancing nuclease-resistance, or binding affinity by providing more target recognition functionality or generating more stable aptamer structures [9,10]. Furthermore, aptamers can be easily tagged with fluorescence reporters, nanoparticles or other functional groups for immobilization on different platforms which make them suitable as biosensor components [11,12].

In this review, we will focus on strategies of using aptamers as affinity ligands of molecules of therapeutic and pharmaceutical interest and as biorecognition part of biosensors for biotechnological and clinical use.

2. Molecular interaction of aptamer with target molecules

Following induction of denaturation and renaturation, aptamers form characteristic three-dimensional structures, such as stems, loops, hairpins, triplexes or quadruplexes. This tertiary conformational structure provides the key for understanding molecular interaction of the aptamer–target complex. Several principal features of interaction mechanism involved in the target recognition have been detailed [2]. Mechanisms for binding affinity, target specificity, thermodynamics and kinetics are significant chemophysical features, which determine how aptamers and target molecules will interact, associate and dissociate.

First, the binding affinity is a significant feature of aptamers. Generally, aptamer–binding affinities are higher with a large size target when compared to small size target molecules. Aptamers selected against protein targets reveal K_d values within pico- or nanomolar concentrations while small molecules targeted aptamers have relatively low affinity to their targets with K_d values in the micro- or mill molar range [3], although there are some aptamers directed against small molecules like tetracycline with nM K_d values [13].

High selectivity of aptamers for their targets was confirmed in many cases. Isozymes of protein kinase C differing in 23 residues were discriminated, being selectively inhibited by aptamers identified against one isozyme [14]. Although the coagulation factor VIIa and factor X share a common structure domain, only the factor VIIa was selectively recognized by modified RNA aptamers, promoting the anti-coagulation effect [15].

As described in the previous section, enantiomer-selectivity has been observed for various aptamers selected against small molecules. Enantiomer selectivity was improved following the use of SELEX protocols, which included a counterselection procedure with the non-cognate ligand citrulline [16]. The recognition of L-arginine was also highly improved by the factor of 10,000 compared to D-arginine when a counterselection procedure had been used.

Small molecules, such as theophylline, FMN and AMP flat aromatic ligands are bound by fitting into the aptamer conformation [17]. Ligand binding involves a planar surface, in which the ligand forms intermolecular hydrogen bonds. In this induced fit model, aptamers fold into a well-defined binding pocket upon association with the target and unfold upon dissociation of hydrogen bonds. For larger targets, such as proteins, the aptamer binding site is induced to fit better into the surface of the target by maximizing complementarity which is driven by noncovalent ligations including hydrogen bonds, electrostatic interactions, base stacking effects and hydrophobicity.

Chemical and thermodynamic mechanisms of aptamer binding have been studied by circular dichroism and isothermal titration calorimetry (ITC) analyses, suggesting that the anti-tyrosinamide DNA aptamer undergoes the enthalpy-driven process as well as the conformational change of DNA from the B-form to A-form in the binding behavior [18]. The same analyses provided evidence that the complex formations between DNA aptamer and L-argininamide involved significant negative enthalpy changes (ΔH), measured as high as −8.7 kJ/mol and the free energy (ΔG) as–5.1 kcal/mol