

Aptamers: an insight for fisheries research

Abstract

Aptamers are oligonucleotides derived from an *In vitro* evolution process called SELEX. Now, with the development of SELEX process, the isolation of small oligonucleotides that possess the capacity to recognize various classes of target molecules have been made much easier. Aptamers have been evolved to bind proteins which are associated with a number of disease states. They are oligonucleotides sequences that have the tendency to bind to a wide range of target molecules, such as drugs, proteins or other organic or inorganic molecules with high affinity and specificity. These molecules offer a tough rivalry to the antibody classes in terms of therapeutic and diagnostic applications. Aptamers exhibit significant advantages relative to protein therapeutics in terms of size, synthetic accessibility and modification by medicinal chemistry. Despite these associated advantages aptamers are not used on a massive scale commercially, the aptamer-based diagnostic research is still in its infancy. With the increasing use of aptamers in numerous diagnostics, therapeutics and other such aspects, it is likely that the perception of nucleic acid therapeutics will be changed and that aptamers may form the basis of future therapeutics in aquaculture research.

Keywords: Aptamers, SELEX technique, Antibodies, DNA, RNA, Yeast, Trypanosome, oligonucleotides, Temperature, Picomolar range, Dyes, Amino acids, Antibiotics, Peptides, Protein, Vitamins

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Abbreviations: SELEX, Systematic Evolution of Ligands by Exponential Enrichment; NMR, Nuclear Magnetic Resonance; VHSV, Viral Haemorrhagic Septicaemia Virus; OMPs, Outer Membrane Proteins; LPS, Lipopolysaccharides

Introduction

“Aptamers” is derived from the Latin word “aptus”, meaning “to fit”. Aptamers are oligonucleotides (DNA or RNA) that have tendency to bind with high affinity and specificity to a wide range of target molecules, viz. drugs, proteins or other organic or inorganic molecules.¹ whole viruses and bacteria and even multicellular organisms such as yeast and trypanosome.² It offers the opportunity of developing affinity-based detection systems.

Antibodies, providing molecular recognition cater a wide range of applications. They have made wide contributions toward the advancement of diagnostic assays and have become indispensable in most diagnostic tests that are routinely used in clinics. Development of the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process however has made possible, the isolation of oligonucleotide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity.

Aptamers are generated from a library of nucleotides or proteins containing approximately more than 1015 different molecules and are selected *In vitro* by SELEX process.³ Great efforts have been made to make aptamers clinically relevant for detection of diseases like viral disease (cancer, HIV) and macular degeneration. Aptamers are highly specific, relatively small sized, non-immunogenic oligonucleotides that have molecular recognition properties and are advantageous over antibodies.⁴

Types of aptamers

- I. DNA or RNA Aptamers: short strands of oligonucleotides.
- II. Peptide Aptamers: short variable peptide domain, attached at both ends to a protein scaffold.

Structure of Aptamers

Aptamers are single-stranded nucleic acids with defined tertiary structures which selectively bind to target molecules. Aptamers also possess ability to bind a complementary DNA sequence to form a duplex structure. The Duplex is formed between a fluorophore labelled DNA aptamers and a small oligonucleotide which is modified with a quenching moiety (denoted QDNA). When the target is absent, the aptamers bind to QDNA, then after it brings the fluorophore and the quencher into close proximity for maximum fluorescence quenching. When the target is introduced, the aptamers prefer to form the aptamer-target complex. The switching of the binding partners for the aptamers occurs in conjunction with the generation of a strong fluorescence signal owing to the dissociation of QDNA. Aptamers are capable to form stable three-dimensional structures in aqueous solution. Single-stranded oligonucleotides follow the same rule of complementarity of nucleic acid bases as do double-stranded molecules, thus it is relatively simple to predict their secondary structure. Different types of secondary motifs of Aptamers are (a) Hairpin (b) Pseudoknot (c) G-quadruplex (d) Three-dimensional representation of an anti-malachite green aptamer in complex with its ligand. The process of folding structures in the solution and the ligand-induced conformational changes were studied to be strongly dependent in the presence of divalent cations. Structures can be experimentally determined by crystallography.⁵ or Nuclear Magnetic Resonance (NMR) spectroscopy (Figure-1).

Aptamers selection

Development of aptamer by SELEX technique enable the selection of specific oligonucleotides from library of randomized molecules. SELEX method has characteristics to identify the unique RNA/DNA molecules, from very large populations of random sequence oligomers (DNA or RNA libraries). Aptamers possess a randomized region of 30–50 nucleotide flanked by constant sequences which helps in PCR amplification. The criteria of SELEX is to stimulate evolution (Systematic Evolution of Ligands). It can

be done with both ways by introducing many rounds of selection (usually 10–15) and by exponential amplification of ligands via PCR after each round (Exponential Enrichment). Finally, the DNA strands are separated and those corresponding to the original library strands are subjected to a next round of selection.² The manipulation of selection parameters (pH, temperature or buffer composition) can be easily done to obtain optimal aptamers. At the initial step, incubation of DNA/RNA sequences from library is done with an immobilized target. Unbound DNA/RNA molecules are washed away and the ligands that get binded are eluted and amplified, either by the process of standard PCR or RT-PCR in the case of RNA aptamers. On the completion of several rounds, the selected molecules are randomly cloned and sequenced. Aptamers have a very high affinity to their targets, with dissociation constants varies from the micromolar to low picomolar range, comparable to those of some monoclonal antibodies, sometimes even better. Single-stranded DNAs also fold *In vitro*, structures forming like stem-loop, internal loops, etc., even if less stable than the corresponding RNA structures.⁶ High-affinity aptamers after being selected bind to hundred different target molecules such as organic dyes,⁷ amino acids,⁸ antibiotics,⁹ peptides,¹⁰ proteins,¹¹ and vitamins.¹² (Figure- 2).

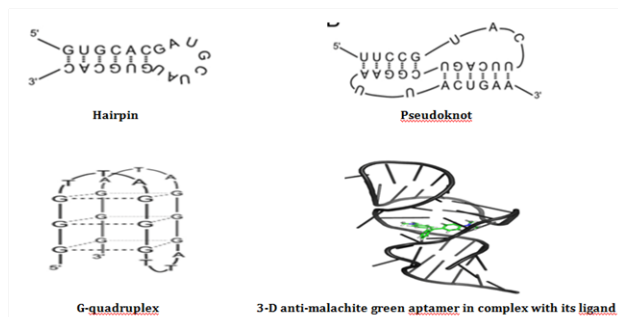


Figure 1 Aptamers secondary structure.²⁰

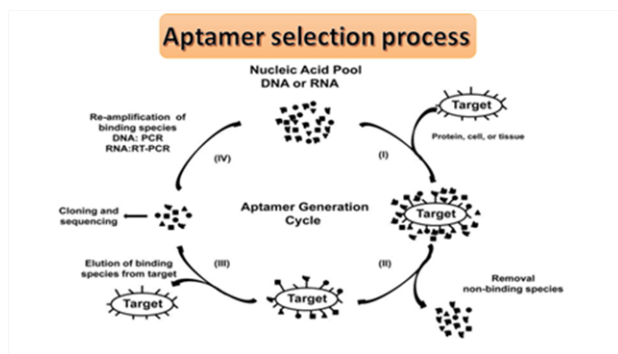


Figure 2 Nucleic acid aptamers: clinical applications and promising new horizons.⁴

In the case of RNA aptamers, they have to be first reverse-transcribed thus an RNA polymerase promoter (usually T7) must be introduced during library construction.² (Figure-3).

High affinity property of aptamers for their targets can be seen by their capability of folding upon binding with their target molecule - they can either incorporate small molecules into their nucleic acid structure or integrate into the structure of larger molecules such as proteins. Aptamer receptors are more advantageous as compared to the antibodies which make them very promising in analytical and diagnostic applications. Most of the antibody production in biological systems by inducing an immune response to the target analyte, but the immune response can be failed also if the target

molecule, i.e. protein, has a similar structure to endogenous proteins. But isolation of aptamers by *In vitro* methods are independent of animals. Generation of antibodies in host means that is the animal immune system is involved which selects the site on the target protein to which the antibodies bind. Whereas aptamer selection process can be manipulated to generate aptamers that bind a specific region of the target and which has specific binding properties in different binding conditions. After selection of aptamer is done, aptamers are produced by chemical synthesis and modifications in the aptamer can be achieved by enhancing the stability, affinity and specificity of the molecules. However, the primary limitation on the use of aptamers (mainly RNA aptamers) as recognition elements has been their nuclease sensitivity which is very critical for their use in *ex vivo* and *In vivo* applications.¹³ By chemical modification of the Ribose ring at the 2-position, the stability of such molecules can be improved. In general, RNA aptamers possess the possibility of intracellular expression, whereas DNA aptamers are more stable. No significant differences in specificity or binding properties have been observed between these two types.

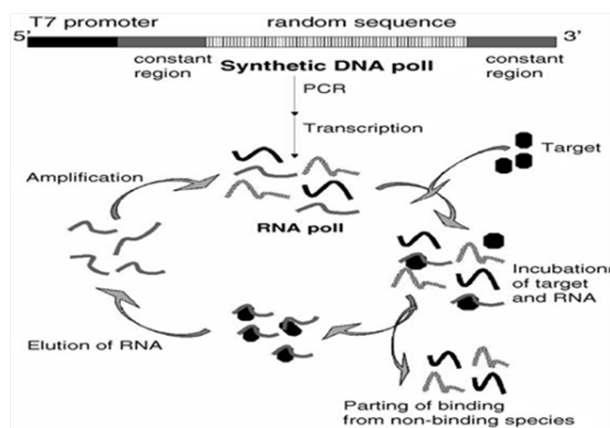


Figure 3 Analytical applications of aptamers.²¹

Application of Aptamers

Food safety control

Food safety plays a very important role in our day-to-day lives because varieties of toxic and harmful substances in food affect human health. Aptamers technique is most promising, which helps to overcome many disadvantages of other existing detection methods in food safety, such as long detection time, low sensitivity, difficult, and expensive antibody preparation. Food borne diseases represent a global public health challenge. Each year, millions of people get sick, and some even die as a result of the ingestion of unsafe food and water. The so-called globalization of the food production, processing and distribution, further complicates the problem. Because of the increase in consumers concerns about what is in their food and its relation with health, control over the safety and quality of food has become tighter, particularly in developed countries where food availability problems are much lower.

Ensuring food safety is nowadays a top priority of authorities and professional players in the food supply chain. One of the key challenges to determine the safety of food and guarantee a high level of consumer protection is the availability of fast, sensitive and reliable analytical methods to identify specific hazards associated to food before they become a health problem. Success in biosensor design depends largely on the development of novel receptors with enhanced

affinity to the target, while being stable and economical. Aptamers fulfil these characteristics, and thus have surfaced as promising alternatives to natural receptors. Therefore, aptamers can be used for the control of pathogens, allergens, adulterants, toxins and other forbidden contaminants to ensure food safety.

Cellular biomarker assay

Cell-SELEX enabled isolation of many aptamers, which recognizes particular cell types. This include range from bacteria through unicellular parasites to mammalian cells.

Medical diagnostics

The perfect sensor used for medical diagnostics ought to be highly specific, sensitive, repetitive, fast and easy to read. It should also be non-reactive and perform well under physiological conditions. Aptamers meet all these requirements. Due to the wide spectrum

of potential aptamer targets, detection of virtually all toxins, drugs and metabolites in body fluids at exceptionally low concentrations becomes feasible.

Electrochemical sensor

Sensors possess higher sensitivity and cost of detection is low as no sophisticated optical machinery is required. Other important application of aptamers are as followings:

- I. Detection of biological terrorist threat agents.
- II. Application to the detection of food borne pathogens.
- III. Aptamers may also used as probes to view tumour cells and might prove very helpful in cancer therapy.
- IV. New drug development, drug delivery, bio-imaging hazard detection, food detection (Table 1).

Table 1 Current Aptamers available for clinical development.⁴

Aptamer	Nucleotide	Target	Condition	Company
Pegaptanib	RNA	VEGF	Macular degeneration	Pfizer/Eyetech
AS1411	DNA	Nucleolin	Acute myeloid leukemia	Antisoma Research
REG1(RB006/RB007)	RNA	Coagulation factor ix	Coronary artery disease	Regado Bioscience
ARC1779	DNA	vWF	Thrombotic thrombocytopenic	Archemix
NUI72	DNA	Thrombin	Heart disease	ARCA Biopharma
NOX-A12	RNA	CXCL ₁₂	Hematopoietic stem cell transplantation	NOXXON Pharma AG
NOX-E36	RNA	CCL ₂	Type 2 diabetes mellitus	NOXXON Pharma AG
ARC1905	RNA	C ₅	Age related macular degeneration	ophthotech
E10030	DNA	PDGF	Age related macular degeneration	ophthotech

Successful application of aptamer in fisheries

In vivo tests using RNA aptamers produced by *Rhodovulum sulfidophilum* showed that extracellular RNA aptamers inhibited VHSV infection in Japanese flounder. These results suggest that the RNA aptamers are a useful tool for protection against VHSV infection in Japanese flounder. Viral haemorrhagic septicaemia virus (VHSV) is a serious disease which has impact on wild and cultured. Hence, a most powerful therapeutic method against VHSV infection is needed to be developed. Aptamer is a novel technology and promising method for diagnostics and therapeutics. Here, it is aimed at selecting RNA aptamers that specifically binds to and inhibits the growth of a strain of fish VHSV both *In vitro* and *In vivo*.¹⁴

Various Aptamers screening technologies summarizes the recent Salmonellae are a significant causative organism of food borne illness worldwide. Selection and evaluation of DNA aptamers were done for the capture and detection of *Salmonella enterica* serovar *typhimurium*. Aptamer sequences were modified against *S. typhimurium* outer membrane proteins (OMPs) with counter-selection against *Escherichia coli* OMPs and lipopolysaccharides (LPS).¹⁵

Future prospects

The virtually infinite array of potential target molecules places aptamers among the most powerful tools in biotechnology. Aptamers possess the potential to improve many of the standard laboratory techniques, which involves purification, detection or concentration measurement of a molecule of choice. Aptamers that have reached the clinical trials drugs can be developed against diseases such as, neo-vascular age-related macular degeneration, diabetic retinopathy, haemophilia, acute myeloid leukaemia, non-small-cell lung cancer, renal cell carcinoma or an acute coronary syndrome. Apart from having

enormous applications, aptamers have not yet been used in aquatic animals. The reason is that there are some limitations that have to be considered for *In-vivo* applications, such as aptamer stabilization, delivery method and mass production.¹⁶ But now several methods have been used to enhance the stability of RNA aptamers, such as amino or fluoro modifications at the 2' position of pyrimidine.¹⁷⁻²¹ These results may lead to a new method for improving the health of aquatic animals.

Acknowledgments

None.

Conflicts of interest

None.

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