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MINI-REVIEW

New Horizons with A Multi Dimensional Tool for Applications in Analytical Chemistry—Aptamer

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ABSTRACT

Enzymes, microbes, antibodies, plant or animal cells/tissues/organelles have been used as biological molecular recognition agents (MRAs) for preparation of biosensors and development of bioanalytical methods. The progress in different technologies has evolved high sensitivity and specificity of MRAs such as enzymes, microbes, antibodies, and new agents like aptamers, and artificial materials (molecular imprints, peptides, nucleic acids, etc). Among these recognition agents aptamers have gained considerable importance and become exciting candidates from the beginning as MRAs. Aptamers can be selected in vitro from a randomized pool of oligonucleotides (10^{14}–10^{15} molecules) by a method called

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Systematic Evolution of Ligands by EXponential enrichment (SELEX). Aptamers with simple structures and easy generation in vitro have been applied in multi disciplines, since they displayed high affinity and selectivity towards the target molecules. The dynamic features of aptamers are highly useful in the development of new bioanalytical techniques, diagnostic tools, and biosensors. This review mainly focused on the advantages, advances, and applications of aptamers.

Key Words: Aptamer; Aptamer beacons; Biosensors; Nuclease resistant nucleic acids; SELEX.

INTRODUCTION

The discovery of deoxyribonucleic acid (DNA) structure by Watson and Crick in 1953[1] was a momentous event in science, which gave rise to entirely new disciplines and influenced the course of many others. The nucleic acids, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA), are the molecular repositories of genetic information. The structure of every protein and ultimately of every cell constituent is a product of information programmed into the nucleotide sequence of a cell's nucleic acids. During the replication and protein synthesis number of specific proteins bind to the nucleic acids very specifically, which is necessary for the progress of biological events in the cell. The relatively simple and repetitive composition of RNA or DNA molecules makes them easy to synthesize and manipulate outside the confines of cells. Along with this the recent advances in in vitro selection and amplification techniques have paved a path for the identification of specific nucleic acid sequences, which bind to the target molecules like proteins, toxins, small molecules, and even whole cells, with high affinity and specificity.[2–5] Such nucleic acid sequences are termed as "aptamers;" in more scientific manner aptamers are RNA or DNA molecules selected in vitro from vast populations of random sequences that recognize specific ligands by forming binding pockets. Aptamers are in the size of 25–80 nucleotides that can be selected in vitro from a randomized pool of oligonucleotides (10^{14}–10^{15} molecules) by a method called Systematic Evolution of Ligands by EXponential enrichment (SELEX). The SELEX process selects the aptamers, on the basis of binding between a target and nucleic acid molecules.

In 1990, the discovery of aptamers by Tuerk and Gold[6] and subsequently by Ellington and Szostak[7] spawned significant interest within academia and industry. Afterwards, within a short span they entered into therapeutic applications[8,9] and emerged as a valuable research tool.[3,10,11] In addition, the environmental, agricultural, and medical analytes and the quickly growing

areas of genomics, proteomics, and metabolomics require the development of high-throughput and massive-parallel analysis of biological samples.[12,13] At this regard, the biosensor technology coupled to aptamers can present an useful approach for the areas of functional genome and advanced molecular biology. Aptamers have been used as sensing/recognizing tools in biosensors[14] and purification processes.[15] In this article, authors comprehensively reviewed the developments, advantages, and applications of the aptamers in detection of analytes.

DISCUSSION

SELEX

The starting point for the generation of a high-affinity aptamer (Latin aptus, suitable or fit) is the synthesis of a nucleic acid library (DNA/RNA) of large sequence complexity, followed by the selection for oligonucleotide, which is able to bind with a target molecule, through a simple procedure called SELEX. Several reviews have described the SELEX procedure in detail.[11,16,17,21] Using SELEX methodology the isolation of aptamers may be very quick and against to different types of molecules like amino acids,[22] peptides,[23] antibiotics,[24,25] dyes,[7] proteins,[2,26] etc. Nucleic acid library is easily obtained via combinatorial chemistry synthesis[27,28] for the selection of aptamer.

To separate the high-affinity nucleic acid ligands against the target molecules, the starting library of nucleic acids (commonly about $10^{14}-10^{16}$ different sequences with a variable region of 30 bases flanked by primers of 20–25 bases) is incubated with the desired and/or target molecules. The rare nucleic acid ligands that bind to the specific target are separated by filtration[19] through nitrocellulose for protein targets or by affinity chromatography,[29,30] generally for small molecule targets. The isolated population of sequences that bind to the target is amplified by RT-PCR or PCR to produce a new lower complex pool enriched in target-binding species that, in the case of RNA libraries, are then transcribed in vitro and used for the next selection/amplification cycle (SELEX cycle depicted in Fig. 1). The efficiency of enrichment of high-affinity binders is governed by the stringency of selection of each round. To obtain high-affinity binders negative selection (removal of aptamers that bind to ligand supports) and counter selection (removal of aptamers that bind to structures similar to those of the targets) are commonly employed.[27] The number of cycles required is dependent on the stringency imposed to each round as well as on the affinity of interaction between the targets and the aptamers. In general, around 8–15 cycles of affinity selection and amplification

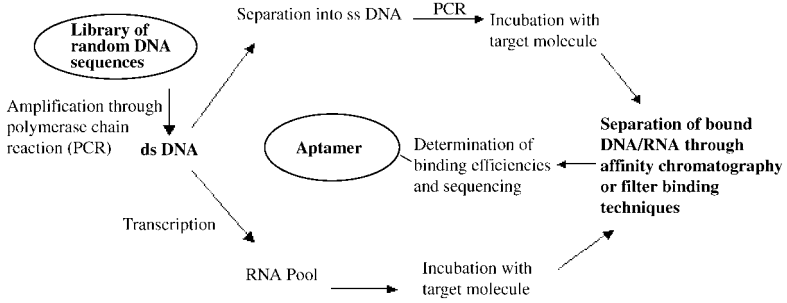

Figure 1. Schematic diagram for the selection of aptamer through SELEX.

are needed before selecting the high-affinity aptamers. Moreover, each aptamer molecule exhibits an unique tertiary structure, which depends on the nucleotide sequence of the target molecule.[31] The binding affinity of the oligonucleotide for the target protein is determined by the fit between moieties on oligonucleotide surface and epitopes on target surface. After selection of high-affinity aptamer the structure and sequence studies are necessary. X-ray and nuclear magnetic resonance (NMR) have been employed for the structural studies.[32–34] The sequences of aptamers have been characterized via standard foot printing and damage selection.[35] These studies are helpful for the detection and deletion of non-binding sequences from aptamers. The analysis of chemical interactions between target and aptamer is also necessary for the construction of high-affinity aptamers and the introduction of reactive groups in certain nucleotides.[36,37]

Importance of Nuclease Resistant Aptamers

Nucleic acids are relatively stable in vitro (in absence of nucleases), but they are unstable in the blood stream.[27,38] The most active RNAase in blood has the same specificity as pancreatic RNAase. Hence, the utility of aptamers is often limited by their vulnerability to nucleases present in biological materials.[39] The most apparent chemical way to stabilize nucleic acids is thiophosphate, rather than phosphodiester bonding.[40,41] Usually nucleases attack the nucleic acids at 2′ position of pyrimidine nucleotides. The change in the 2′-OH group of ribose to 2′ amino or 2′ fluro of pyrimidines provides resistance to nuclease attack in serum[42–44] and substantial stability up to 15 hr instead of 10–15 sec.[14,27,45]

The short lifetime of anti-thrombin DNA aptamer has encountered due to the blood nucleases and rapid clearance from the blood through excretion

organs like kidney.[38] Blood nuclease assays and biodistribution analyses were carried out in mouse and rabbit to study the stability of modified DNA aptamers. Modifications were done at 3′ by conjugating with biotin and biotin steptavidin. 3′-Biotin protected the aptamers significantly from blood nuclease in vitro, while it did not show in vivo clearance. However, the 3′-biotin–SA bio-conjugates were resistant to the blood nuclease in vitro and showed a longer life (10–20 times) in blood circulation without excretion.[38] In another study, the instability of anti-vesicular endothelial growth factor (VEGF) RNA aptamer was observed. Improved nuclease resistance has been obtained by substituting 2′-*O*-methylpurine nucleotides at 3′ and 5′ ends at all purine positions, and 2′ position of pyrimidines has been modified with amino groups without loss of binding affinity.[46] Whereas, the residence time of aptamer in the blood stream was found to be very short due to the renal excretion, because the aptamer was small in size (~7–12 kDa). To improve the residence time liposome-associated (~50–65 nm dia.) VEGF aptamers were prepared.[47]

Mirror image aptamers have been developed for the improvement of aptamer stability. D-DNAs are highly susceptible to the nuclease attack, while L-DNA forms are insusceptible. Mirror image single-stranded DNA (L-ssDNA) that binds to the peptide hormone vasopressin has showed good stability to nuclease attack and good bioactivity as a vasopressin antagonist in biological fluids. L-Aptamer is stable up to 10 days, however, D-aptamer is degraded within a period of 10 sec in the presence of nucleases. Both aptamers are degraded in the presence of calf serum. The half-life for L-aptamer per nucleotide is about 2 days, but in presence of human serum it is highly stable.[39]

Aptamer Probes

Selective and specific probes are extremely important for the real time monitoring of compounds in clinical diagnosis and living cells, for their concentration, location and co-localization, and secondary modifications of the large sets of proteins and other target molecules. In order to develop an effective detection method or system in addition to molecular recognition elements, good signaling compounds/probes are highly necessary. The signaling compound should be stable, easy to conjugate, and sensitive even at low concentrations. One of the best approaches is the development of molecular aptamer beacon (MAB),[12] with which a real time target recognition and quantification is possible. MABs are similar to the molecular beacons (MBs).[48] Like MBs the 5′ and 3′ ends in MABs can also be tagged with fluorphore and quenching molecules, respectively (Fig. 2). The presence of target molecule results in a

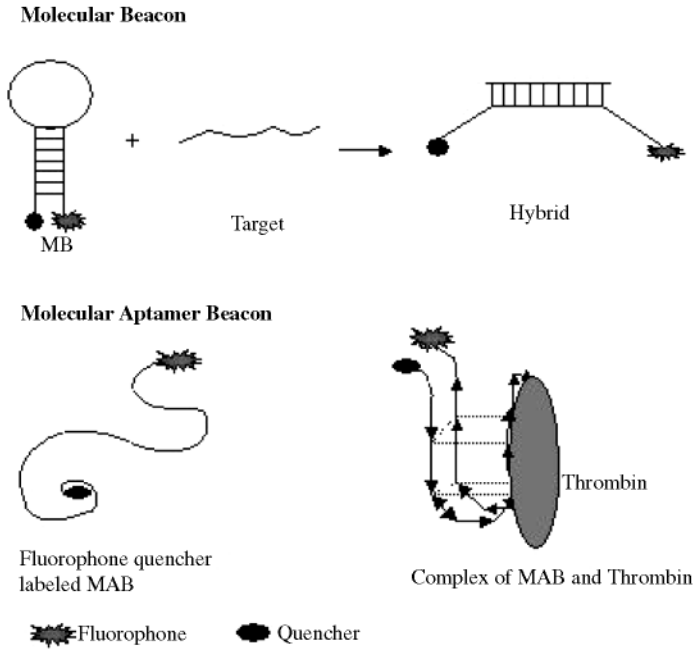

Figure 2. Structures of MB and MAB. (Adopted from Ref. 12).

conformational change and/or a distance increase between quencher and fluorophore, which leads to detectable signal. The fluorescence signal is directly proportional to the target concentration or may be quenched.[12] Li et al.[12] constructed an anti-thrombin aptamer beacon to recognize thrombin. The aptamer showed high specificity and sensitivity with a detection limit of 112 pM in homogeneous solution.

Usually, the fluorescent and radio labeled antibodies are used for the in vivo quantification or detection of target ligands. These methods require large aggregates of target ligands for the detection. The unbound antibodies also produce fluorescence, which interferes the detection. MABs produce a signal when they specifically bind with a target molecule, thus they will facilitate the in vivo quantification of target ligands very specifically. To develop a MAB-based biosensor, Stojanovic et al.[49] selected an anti-cocaine DNA aptamer with a binding affinity of 200 μM through SELEX and affinity purification. The aptamer was labeled at 5′ with a 6-carboxy fluorescein and 3′ with dabcyl quencher. Using this aptamer probe a wide detection range of 10–150 μM was obtained for cocaine. In another study MAB was constructed using anti-rATP aptamer labeled with rhodamine and dabcyl.[50] The sensitive

detection range for this aptamer was from 10 μM to 1 mM. However, the fluorescence responses of fluorescein and acridine are low in the detection of target ligands.[50] To improve the fluorescence response Yamana et al.[51] labeled the anti-ATP aptamer with *bis*-pyrine fluorophore. This fluorophore showed high response towards the detection of ATP and was easy to conjugate with aptamer into internal or terminal position. The reagentless detection systems are more convenient for in vivo metabolism studies. A mutant of *E. coli* phosphate binding protein labeled with a fluorescent dye adjacent to its binding site exhibited a large increase in fluorescence upon inorganic phosphate binding.[52] The same kind of labeling studies were conducted for the determination of maltose[53] and glucose.[54] With a similar way anti-adenosine aptamer was modified to yield a specific ATP dependent increase in fluorescence.[55] This probe could be used for in vivo detection of ATP up to a concentration of 25 μM.

The rare cells or target molecules can be separated through magnetic separation/sorting using magnetic probes, *i.e.*, aptamer–magnetic particle conjugates. In this process the conjugated aptamers and target molecules are incubated with efficient mixing, finally, in magnetic field target ligands/cells can be separated. Magnetic separation is quick, easy, and economic, and can achieve automation compared with other laborious purification processes. Aptamer enzyme conjugates can be effectively utilized for amperomertic and colorimetric detections of target molecules.[56,57] In future there will be a great need for construction of new aptamer probes/conjugates for the sensitive and precision detections.

Advantages of Aptamers over Antibodies

Antibodies have made a substantial contribution towards the advancement of diagnostic assays and have become indispensable in most diagnostic tests that are used routinely in clinics today.[4] The development of SELEX process made the aptamers possible to be used as potential substitutions for the antibodies and other receptor molecules. Aptamers may mimic antibodies in a number of applications,[4] such as flow cytometry,[58] enzyme linked immunosorbent assay (ELISA),[59] immuno bead assays,[60] cell sorting,[61] fluorescence microscopy,[62] western blotting,[63] and biosensors or chips.[7,64,65] Aptamers have number of advantages compared to antibodies in several dimensions. They are smaller in size, the sensitivity can be increased, possibility for in vitro production, no need of immunization and animal hosts.[9,10,66] The binding affinity, specificity, and stability of aptamers can be improved by rational design or molecular evolution techniques.[9] They are highly specific towards the targets, for example, caffeine has a 10,000-fold lower affinity

than theophylline, which differs only in one methyl group.[67] The aptamers can bind selectively with wild-type thrombin without a variant of thrombin differing by just one amino acid[68] and are able to discriminate very specifically L- and D-amino acids.[22] The functional groups of aptamer can be altered for the directly or indirectly covalent immobilization on biochips or other support materials, resulting in highly ordered receptor layers. However, the generation of the same affinity and avidity antibodies is difficult. The homogeneous quality of aptamers can be developed via chemical synthesis.

Antibodies are large molecules. Their complex systems and paratopes (antigen-binding sites) are sensitive to the acidic and basic exposures for elution during the regeneration and affinity purification. Regeneration of aptamers is easier than antibodies, due to the simple and quite stable structure, which is a highly important and key factor for the repeated use, economy, and precision of biosensors and affinity columns. Antibodies are temperature sensitive molecules when compared with aptamers. In the ELISA immunoglobulins are primary recognition agents; using the same principle enzyme linked oligonucleotide assay (ELONA) can be formatted using aptamers as recognition agents. Different ELONA formats have been well described by O'Sullivan[27] and Ito et al.[69] An ELONA process was patented by Drolet et al.[70] Lyophilized aptamers can be stored for years without loss of activity, and once they are reconstituted aptamers can be subjected to numerous freeze and thaw cycles.[71]

Applications of Aptamers

Several reports have described the use of biomolecules such as enzymes,[72–75] antibodies,[76–80] tissues,[81] and whole cells[82–84] as the biosensing agents in various disciplines for numerous applications. Aptamers have been used widely since the last decade in the fields of therapeutics,[9,85–87] biosensors,[88] separation and purification[71,89] because of their extraordinary flexibility.

Therapeutic

Aptamers can block the receptors and inhibit protein activities with high affinity and specificity, hence they have been widely used in therapeutics since starting. The ideal aptamer should be ascribed at least six properties for the therapeutic use: high affinity and specificity for the target molecule, rapid uptake in the target tissue, rapid blood clearance, urinary excretion, durable tissue retention, and accumulation of high concentration in the target tissue. In recent years aptamers have been widely used in various therapeutic

applications like target validation and drug screening, due to their small size, quick elimination of small oligonucleotides, low production cost, biocompatibility, biodegradability, and no cross reaction with antibody-binding receptors in vivo. Certainly aptamer molecules should be modified before being used as a therapeutic agent, because blood is rich in nucleases, which can degrade the nucleic acids.[14] However, appropriate modification of single-stranded RNA/DNA can make them susceptible to nuclease attack.

Rusconi et al.[90] reported a RNA aptamer that inhibited the factor VIIa in the blood. The tissue factor or factor VIIa complex is a primary initiator for the most physiologic blood coagulation events. They employed combinatorial RNA library and in vitro selection methods for the isolation of high-affinity and nuclease-resistant RNA ligand. This RNA aptamer significantly prolonged the prothrombin time of human plasma in a dose dependent manner, and dramatically improved the half-life, approximately 15 hr in human plasma. This RNA ligand represented a novel class of anti-coagulant agents directed against factor VIIa.

An aptamer-based inhibitor, against human complement C5 specific, has been isolated through SELEX methodology.[91] After 12 cycles of iterative binding and nucleic acid amplification a pool of high-affinity binders to the C5 protein were obtained. Seven of these aptamers bound to C5 with dissociation constants of 20–40 nM and also inhibited human serum hemolytic activity. These aptamers also inhibited zymosan-induced generation of C5a. Affinity of this aptamer was improved by performing a second biased SELEX process to obtain a better affinity with dissociation constants of 2–5 nM. The human and rat aptamers were evaluated for complement inhibition in vitro and in vivo as potential therapeutics for treatment of human diseases related to hemolytic activity. Usually the reverse transcriptase (RT) of HIV is essential for viral replication. Anti-RT drugs such as AZT block viral replication, but their clinical effectiveness is limited by the rapid appearance of resistance mutations. The binding of aptamers to HIV RT makes numerous contacts with the proteins and inhibits the activity of HIV RT. This kind of aptamer is an alternative to small-molecule drugs in the form of anti-viral gene therapy. Donald et al.[92] reported a RNA aptamer against RT for the therapeutic application of HIV. In another study, a RNA aptamer, which inhibited the maturation of Hepatitis C virus, was identified by Fukuda et al.[276] through SELEX. This aptamer specifically bound with a nonstructural serine protease with a binding constant of 10 nM and inhibited the 90% enzymatic activity.

Leva et al. [93] identified a DNA and RNA Spiegelmers that bound to the peptide hormone gonadoliberin (gonadotropin-releasing hormone, GnRHI) with high affinity and high specificity. These aptamers bound to D-GnRH with dissociation constants of 50–100 nM. GnRH is a decapeptide molecule that binds gonadotrophic cell receptors on the pituitary gland and initiates

secretion of gonadotropin—lutenizing hormone (LH) and follicle-stimulating hormone (FSH). These, in turn, stimulate the production of sexual hormones in a process, which may lead to certain diseases, including malignant breast and prostate cancers. Currently cetrorelix is widely used as a GnRH receptor antagonist. These DNA and RNA aptamers may be potential alternatives for the present practicing drugs with more efficiency for the treatment of above-mentioned disorders. In another study, a high-affinity RNA aptamer was reported for the detection of African trypanosomiasis caused by *Trypanosoma brucei* protozoan.[94] Commonly this protozoan transmits via tsetse flies and multiplies in the blood of many mammals. The RNA aptamer was isolated against whole parasite and bound specifically to a single 42 kDa protein located within the falgellar pocket of parasite. This RNA aptamer could not discriminate different trypanosome variant strains, but could selectively bind to the flagellar protein as a marker for the diagnosis and development of drugs against African trypanosomiasis.

A nuclease-stabilized anti-tenascin-C aptamer was reported by Hicke et al.[95] Tenascin-C is an extracellular matrix protein that is over expressed during tissue remodeling processes, including tumor growth. This aptamer exhibited a dissociation constant of 5 nM. This novel aptamer ligand may be used in targeted drug delivery to the diseased tissues. For the regulation of HCV polyprotein production an anti-nonstructural protein RNA aptamer was selected through SELEX.[96] This aptamer inhibited the activity of trypsin like protease of nonstructural protein (NS3) for the control of polyprotein secretion in HCV. The targets for currently practicing HCV drugs are also NS3. This aptamer showed selective binding to NS3 with high affinity.[96] Some other therapeutic applications are given in Table 1.

Biosensors

Biosensor is a probe that integrates a biological component, such as whole bacterium or biological product (e.g., tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc), with an electronic component to yield a measurable signal. Biosensors are hailed, as a hallmark in analytical techniques with an ability to constantly identify and quantify desired molecules, rapidly and cost-effectively. They have been applied in medical, environmental, industrial, and defense sectors. In medical field, biosensors are widely used for research purposes, home diagnostics, and clinical diagnostics. In view of the advantages and simple structure of aptamers, they have been used as biological sensing agents in the development of biosensors. A flexible thrombin biosensor has been developed by Potyrailo et al.[107] An anti-thrombin DNA aptamer was fluorescently labeled and covalently immobilized on a glass support. Thrombin in solution was selectively detected by the

Table 1. Therapeutic applications of aptamers.

Target molecule	Type of aptamer	Mode of action	References
Human complement C5 Protein	RNA	Inhibition	[91]
Autoimmune myasthenia gravis	RNA	Inhibition of autoimmune disease	[97]
Monocyte chemoattractant protein-1	RNA	Reduce the inflammatory diseases and atherosclerosis	[31]
Amyloid peptide	RNA	Alzheimer's disease diagnosis	[98]
Hepatitis C virus polymerase	RNA	HCV polymerase inhibitor	[99]
Thrombin	DNA	Thrombin inhibitor	[100]
Taq polymerse	DNA	Inhibitor, could not bind higher than 40°C	[101,102]
Platelet derived growth factor (PDGF BB)	DNA	Cytokine growth and differentiation promoting factor. Detection and analysis	[103]
ATP	RNA	Fluorescence detection, estimation	[104]
E2F protein	RNA	Inhibition effect on E2F protein transactivation in human cells	[105]
G-protein-coupled receptor (GPCR) protein	RNA	Neurotensin therapy	[106]
Gonadotropin-releasing hormone I (GnRH)	RNA and DNA	GnRH inihibitor	[93]

change in the evanescent-wave-induced fluorescence anisotropy of the immobilized aptamer. This biosensor could detect as little as 0.7 attomol of thrombin in a 140 pL interrogated volume with a dynamic range of three orders of magnitude and a total analysis time of 10 min required for each sample. Myoyong et al.[108] reported a fiber-optic biosensor using a DNA aptamer as receptor for the determination of thrombin. An anti-thrombin DNA aptamer was immobilized on the surface of silica microspheres, and these aptamer modified beads were then distributed in micro wells on the distal tip of an imaging fiber. The aptamer modified beads selectively bound to the target and could be reused without any change in sensitivity. This fiber-optic microarray system had a

detection limit of 1 nM for non-labeled thrombin, and each test could be performed within 15 min including the regeneration time.

Detection and inactivation of biological warfare and biological terrorist threat agents and diagnosis of newly emerging diseases are significant in military and civilian challenges. The traditional immunological techniques, including immunomagnetic-electrochemiluminescence (IM-ECL) method,[109,110] have been used extensively by the military for biological warfare agent detection. However, immunological methods depend on antibodies from animal hosts and involve a tedious process. In view of the importance for the detection of anthrax spores, Bruno and Johnathan[111] developed a high-affinity DNA aptamer against inactivated spores. They employed an aptamer–magnetic bead-electrochemiluminescence (AM-ECL) sandwich assay scheme for detection of anthrax spores. This method might be useful for military, civilian, and veterinary concern in near future.

Yamamoto et al.[112] developed a RNA aptamer, which could effectively bound with Tat protein of HIV. A MB[113] was constructed by attaching the fluorophore and quencher at the 5′- and 3′-ends, respectively, for the biosensor development. The properties, binding affinities, structural details, and inhibition mechanism of the Tat RNA aptamer were well studied. Thomas et al.[114] developed a chip-based biosensor for multiplex analysis of protein analytes using anti-thrombin, bFGF, IMPDH II, and VEGF aptamers. DNA and RNA aptamers were selected against different protein targets, for simultaneous detection of individual proteins in complex biological mixtures. The fluorescently labeled aptamers were immobilized on a streptavidin-coated glass via biotin covalent linkage. In this work fluorescence polarization anisotropy was used for solid- and solution-phase measurements of target protein binding. Studies were carried out for the detection and quantification of cancer-associated proteins like inosine monophosphate dehydrogenase II (IMPDH II), VEGF, and basic fibroblast growth factor. Diagnosis of cancer may be faster using the aptamer chips by detecting the closely related proteins. The integration of aptamers with different sensitive measurement techniques such as piezoelectric, surface plasmon resonance (SPR), potentiometric, amperometric, etc. can be used to develop very high-sensitive and real time analytical methods. Most of the research on aptamers are going on towards therapeutic and diagnostic applications. Further research is required for the utilization of aptamer unique features in bioanalytical and analytical disciplines.

Purification

Affinity chromatography is one of the most powerful techniques among the purification methods for rapid selection and separation of proteins or small

molecules from the complex mixtures. Although many affinity interactions can be used, antigen–antibody interactions are perhaps the most common because of their high selectivity, affinity, and the fact that various molecules can be targeted. Although antibodies can be conveniently used for preparation of stationary phases, a number of constraints limit their application in affinity chromatography. Their large molecular size limits the surface loading. There are potential possibilities for antibody degradation and denaturation, the difficulties associated with the immunization for small compounds or non-immunogenic compounds, the difficulty in tailoring the selectivity, and the legal problems from the governments in use of animals. As a result of these limitations, research has been pursued for better alternatives to antibodies for highly selective stationary phases. It has been identified that one of the best promising approaches/tools was the use of nucleic acid ligands, i.e., aptamers. The secondary structures of aptamers make them powerful for preparation of useful stationary phases for separation of target compounds.[115] A high-affinity aptamer has been used by Romig et al.[116] for purification of L-selectin. Selectin was effectively bound to the column. The initial purification step resulted in a 1500-fold purification with a single-step recovery of 83%. This report indicates that oligonucleotide aptamers can be effectively used as affinity purification reagents for stationary phase preparation.

Adenosine and analogues were successfully purified by Deng et al.[89] using DNA aptamer as a stationary phase reagent. A biotinylated-DNA aptamer (MW 16,600) was immobilized covalently through streptavidin, on porous chromatographic supports. An affinity chromatography column was prepared by packing the immobilized aptamer into the fused silica capillary column (50–150 μm i.d.). This column possessed the dissociation constants of 138 ± 18, 58 ± 2, 38 ± 2, 28 ± 6, and 3 ± 1 μM for cyclic-AMP, AMP, ATP, ADP, and adenosine, respectively. Similar values were obtained for aptamer immobilized on a polystyrene support, except a slightly higher dissociation constant for adenosine. It was also reported that the column was able to selectively retain and separate cyclic-AMP, NAD^+, AMP, ADP, ATP, and adenosine for the development of new bioanalytical methods/sensors, even in complex mixtures such as tissue extracts.

DNA aptamers have been successfully used as stationary phase reagents in separation of amino acids and polycyclic aromatic hydrocarbons.[115] G-Quartet structure of DNA was covalently attached to fused-silica capillaries for capillary electrochromatographic (CEC) separation of binary mixtures of amino acids (D-trp and D-tyr), enantiomers (D-trp and L-trp), and polycyclic aromatic hydrocarbons (naphthalene and phenanthrene, benzo[*a*]pyrene, or benzo-[ghi]perylene).[115]

Michaud et al.[71] reported for the first time the use of an immobilized DNA aptamer as a new target specific chiral stationary phase

for high-performance liquid chromatography. Anti-D-vasopressin-specific DNA aptamer was immobilized on a highly porous polystyrene–divinylbenzene support via a biotin–streptavidin bridge. This column showed high stereospecificity, binding capacity, and was stable without change in the retention time for a storage period of more than 5 months. This novel chiral selector would be applied in various fields such as enantioselective solid phase extraction, binding assays, and sensors by paying more efforts.

CONCLUSIONS

For the quickly growing areas in molecular biology, environmental problems, newly emerging diseases, drug discovery and validation, biological weapon threats, highly sensitive, selective, and quick detection, and quantification methods are required. There is a great need for new molecular recognition tools with high affinity and specificity. Aptamers have emerged as very attractive and potential tools in therapeutic and diagnostic applications since their discovery. Within no time they have become rival molecules to the antibodies and other receptor biomolecules. Aptamers are different from antibodies, but mimic properties of antibodies in a variety of diagnostic formats and other applications. Compared to antibody technologies aptamer research is still at juvenile stage, hence lots of efforts are further required to utilize the potentiality of aptamers. It is also likely that existing diagnostic formats may be changed due to the unique properties of aptamers. Using aptamers the miniaturization, integration, and automation are easier and highly convenient for the development and applications of biosensors. New immobilization techniques are highly required for the construction of new biochips/biosensors and the development of new purification methods. Compared to other biorecognition molecules (e.g., enzymes and antibodies) aptamers are very small in size with 30–100 nucleotides including flanking regions. More efforts are being needed for wide therapeutic applications. The potential of aptamers may be realized in near future in the form of aptamer-based diagnostic products, biosensors, etc. in the market.

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