ORIGINAL RESEARCH PAPER

A Biotechnological Perspective on The Affinity Magnetic Separation and Purification Based on Oligonucleotides

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ABSTRACT
The rapidly growing field of biotechnology has created a critical need for simple, fast and high-throughput processes for the separation and purification of biomolecules from biological matrices. In recent years, several bioseparation techniques have been proposed as advanced alternatives to the classical separation methods. These modern processes emphasize ultra-high selective and sensitive analysis to determine promising chemical and biological entities. The current paper discusses the recent developments in the field of biotechnology using magnetic separation techniques based on oligonucleotides as the chemically synthesized and cost-effective biological ligands. In particular, they are very stable and not subject to thermal and chemical degradation. This allows the researchers to use labeled aptamers as highly sensitive and specific olgonucleotide probes in solution, membrane or magnetic nanoparticles-based assay systems. Since aptamers bind their targets with high affinity and specificity, they are promising alternative ligands in the purification of proteins. The purpose of this review paper is to show the critical role of oligonucleotides ligands for separation or purification of biological compounds and ions in complex matrices. Using the suitable aptamers in separation and preconcentration processes is an excellent alternative to the classical ligands. Some practical examples of DNA-based magnetic separation processes are also discussed to show the efficiency of magnetic bioseparation.

Keywords: *Affinity separation; Magnetic nanoparticles; Oligonucleotides*

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INTRODUCTION

Separation technology is an important area for further developments in bio-oriented research. Currently, the new separation methods, capable of treating solutions containing only ultra-trace amounts of target in the presence of vast amounts of accompanying compounds are essential. In the field of biotechnology, the isolation and separation of biomolcules are usually performed using a variety of chromatography, electrophoresis, ultra-filtration, precipitation and magnetic separation being one of the most important techniques.

In recent years, magnetic separation methods in biotechnology have diversified, leading to a wide range of different particles, affinity mechanisms and processes. These techniques have several advantages in comparison with standard separation

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procedures. This process is usually very simple and non-time-consuming. There is no need for tedious solid phase extraction (SPE) *cartridges*, centrifuges, filters or other equipment. Due to the magnetic properties of magnetic sorbents, they can be relatively easily removed from the sample solution with an external magnetic field [1, 2]. However, the conventional magnetic sorbents in many cases exhibit non-specific bindings in complex matrices, leading to reduction of selectivity. To overcome this limitation, affinity magnetic separation as a very unique tool in biochemistry, clinical chemistry, environmental testing and pharmaceutical science is recommended.

Affinity magnetic separation has been widely used for quick and efficient purification and analysis of compounds from complex biological samples with minimal nonspecific adsorption [3]. It is based on a highly specific interaction between bioreceptor and its target. In general, affinity magnetic separations can be performed in two different modes. In the direct method, an appropriate affinity ligand is directly coupled to the magnetic particles or biopolymer exhibiting the affinity towards target compound(s) is used in the course of preparation of magnetic affinity particles. These particles are added to the sample and target compounds then bind to them. In the indirect method the free affinity ligand is added to the solution or suspension to enable the interaction with the target compound. The resulting complex is then captured by appropriate magnetic particles [4]. The two methods perform equally well, but, in general, the direct technique is more controllable. The indirect procedure may perform better if affinity ligands have poor affinity for the target compound.

There is a growing interest for usage of affinity ligands in biorecognition area. A large diversity of ligands is available today for the separation of biomolecules. Antibodies, nanobodies from camelides, peptides, lectins and nucleic acid aptamers are the most known affinity ligands [5, 6]. Aptamers are single-stranded synthetic oligonucleotides that are able to capture their target molecules with high affinity and specificity [7]. They are being an alternative to antibodies owning to the superior advantages over them, including flexibility, thermal and chemical stability, easy artificial synthesis, specificity, low cost and reusability [8, 9]. In addition, aptamers can bind to a broad range of targets, including small molecules [10], ions [11] proteins [12], cells [13], tissues and organisms [14]. Consequently, they can be used in different applications based on molecular recognition including affinity separations [15]. New technologies in the fields of aptamer selection and synthesis are expected to further promote the use of aptamers as affinity ligands in the biomagnetic separation (Fig. 1).

This review summarizes possibilities affinity magnetic separation techniques based on oligonucleotides using the modified magnetic particles and highlights advantages of these methods. The efficiency of affinity magnetic separation is shown in several successful applications, especially in the field of aptamers.

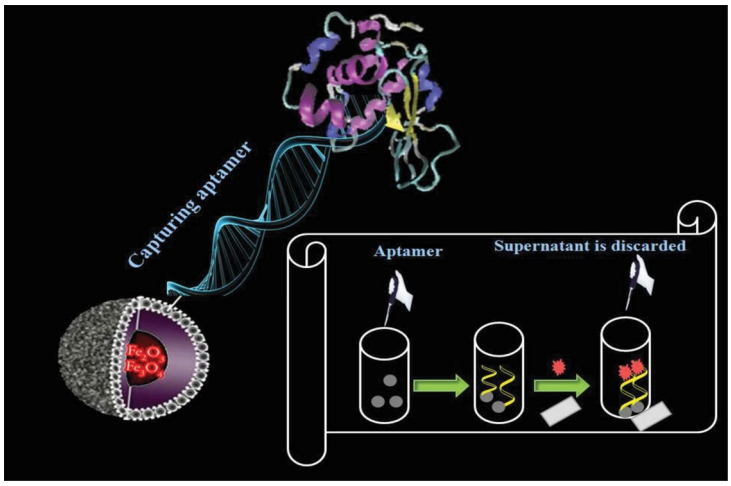

Fig. 1. Affinity magnetic separation based on aptamers.

Magnetic particles

In the domain of biological magnetic separation, there is a high demand for novel magnetic materials with the ability to interface with biological media. Magnetic materials are classified as five main types [16] (Fig. 2). (I) Paramagnetic materials are slightly attracted by a magnetic field and the material does not retain the magnetic properties when the external field is removed. (II) Superparamagnetic materials are nonmagnetic in the absence of an external magnetic field, but they develop a mean magnetic moment in an external magnetic field. (III) Ferromagnetic materials with permanent mean moment such as Fe, Ni and Co. A ferromagnetic material produces a magnetic field, even in the absence of an external magnetic field. (IV) Diamagnetic materials (such as Cu, Ag, Au, and most of the known elements), atoms have no unpaired electrons, resulting in zero net magnetic moment. These materials display a very weak response against the applied magnetic field due to realignment of the electron orbits when a magnetic field is applied. They do not retain magnetic moment when the magnetic field is removed. (V) Anti-ferromagnetic materials (such as MnO, CoO, NiO, and $CuCl_2$) are compounds of two different atoms that occupy different lattice positions. The two atoms have magnetic moments that are equal in magnitude and opposite in direction, which results in a zero net magnetic moment.

Considering the important role of magnetic materials in the field of magnetic separation, possible perspective and some challenges in the further development of them are discussed. It should be also noted that ferromagnetic materials possess stronger magnetic potential than their supermagnetic counterparts, which is attractive for magnetic separation.

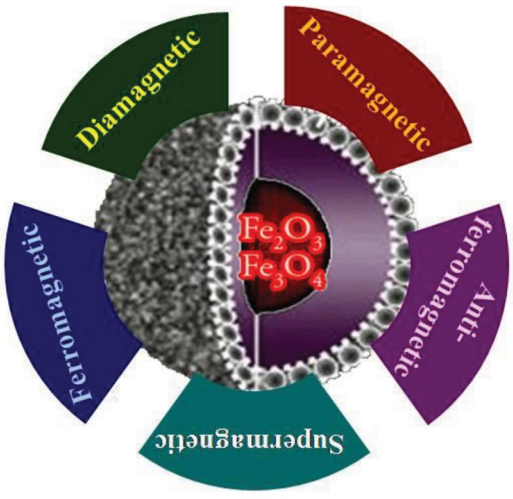

Fig. 2. The main types of magnetic materials.

Magnetic iron oxide nanoparticles

Often the magnetic materials are colloidal in nature (dispersions of particles 1-1000 nm) and exhibit remarkable properties of scale. Iron oxide nanoparticles (IONPs) are one of the most ground-breaking colloids. They have been widely researched for magnetic resonance imaging (MRI), as they are mainly superparamagnetic [17]. In addition, they exhibit some attractive properties such as biocompatibility and biodegradability. When IONPs release in the body, the free irons are integrated in the iron stores of the body, used for metabolic processes, and eventually eliminated from the body [18].

Despite the existence of several iron oxide polymorphs, only magnetite (Fe_3O_4) and its oxidized form; maghemite (γ-Fe_2O_3) have been found to be functional and promising candidates in biochemical and biotechnological applications [19]. In fact, magnetic iron oxide (Fe_3O_4 and γ-Fe_2O_3) NPs have attracted much attention and are especially interested in magnetic bioseparation applications, due to their lower toxicity compared the others polymorphs, favorable superparamagnetic properties, easy separation under external magnetic fields, large surface area and simple preparation [20, 21]. The described features make these supermagnetic NPs less prone to aggregation than their ferromagnetic counterparts, so they have been extensively used as magnetic sorbents and, more recently, for hyperthermia treatment of cancer [22].

The magnetite nanoparticles as the most widely used magnetic nanoparticles are easily oxidized to form of γ-Fe_2O_3 in air or water and can also suffer degradation phenomena when exposed to harsh environments. They are inherently unstable over prolonged periods of time due to their large surface area to volume ratio and low surface charge at physiological pH, so they tend to aggregate [23]. To overcome these drawbacks, they are often coated with protective organic or inorganic layers such as polymers and surfactant stabilizers, precious metals, silica, etc [24]. Furthermore, the combination of graphene oxide (GO) with Fe_3O_4 NPs leads to magnetic nanocomposites with interesting properties for a variety of applications, especially, magnetic separation and preconcentration. Some of the GO properties such as high surface area, ease of functionalization and good dispersibility due to the presence of the oxygen functionalities make it an ideal sorbent. Fe_3O_4/GO nanocomposites

combine the beneficial magnetic properties of the core and the possibility of binding to their surface [25]. In recent years, significant progress has been achieved in the synthesis of magnetic core-shell nanoparticles especially Fe_3O_4@Au NPs [26]. The latest advances in synthetic routes have encouraged the recent use of Fe_3O_4@Au NPs for aptamer-based bioassays. The high surface area of these magnetic nanoparticles along with the gold coating allow the easy immobilization of most biological molecules through several binding strategies, enabling the achievement of optimum loading to maximize the sensitivity of the bioassay [27, 28]. So, the modification of magnetic nanoparticles is unavoidable for most applications and frequently advantageous because it provides the means to functionalize the nanoparticles. The modified Fe_3O_4 NPs can thus show enhanced properties and functionalities that promote biocompatibility. Fig 3 represents coatings of Fe_3O_4 NPs with nanomaterials and metallic films for conjugation of them with functional oligonucleotides.

Magnetic beads

Among the various types of magnetic materials, the magnetic-polymer composites represent a class of functional materials where Fe_3O_4 NPs are embedded in polymer matrixes. The preparation of agarose-polyacrylamide beads as the first magnetic beads was described by Guesdon and Avrameas in 1997 [29]. Later beads have been made of cellulose, starch, agarose, and a large panel of different beads has been produced by the polymerization of either methacrylates, acrylates or styrene [30]. However, the most of these beads did not act uniformly in a magnetic field because of differences in magnetism and in bead size. The breakthrough in producing beads with a highly uniform size came in 1979 when Ugelstad succeeded in making spherical monosized particles in a size range from 0.5 to 100 microns [30]. Currently, magnetic monosized polymer beads are prepared by in situ formation of Fe_3O_4 inside polymeric compounds such as polyvinylpyrrolidone, chitosan and polyaniline [31]. The polymer component stabilizes the magnetic particles and offers swelling ability and elasticity to the beads. Also, polymer endows the particles with functional groups necessary for the desired applications. For example, functionalization of the surface of the beads by specific ligands makes their usage possible in the separation and purification of nucleic acid sequences, cells, microorganisms and also treatment of environmental and biological samples.

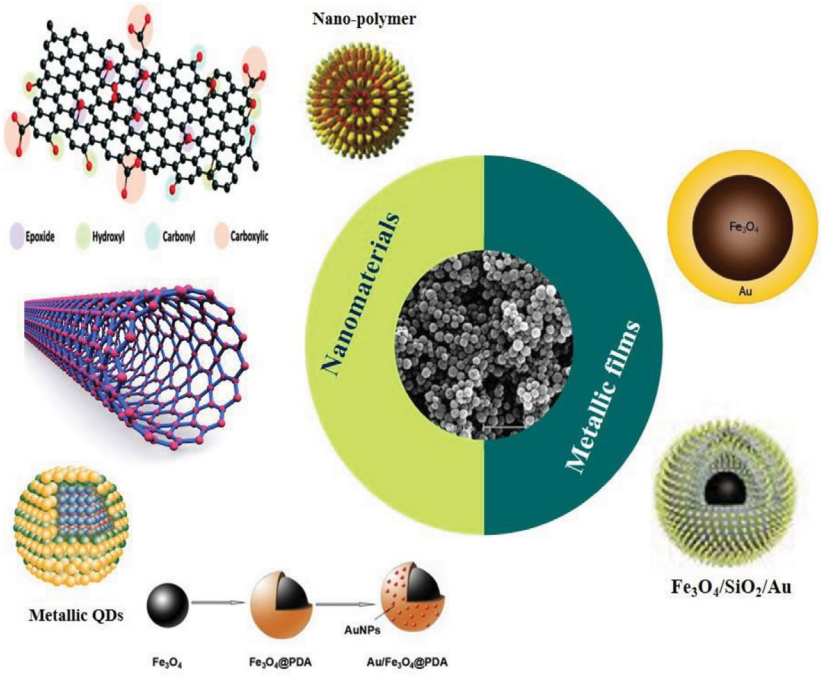

Fig. 3. Surface modification of Fe_3O_4 NPs with nanomaterials and metallic films.

Turbobeads

Turbobeads were developed in 2007 as the next generation of magnetic beads [32]. They are ferromagnetic pure metallic nanoparticles with a core/shell structure. These carbon coated cobalt nanoparticles (C/Co) show a substantially higher saturation magnetization compared to the often used superparamagnetic Fe_3O_4 NPs [33], making them a promising candidate for magnetic separation applications. In addition, turbobeads offer many advantages over the magnetic beads such as higher magnetic saturation, faster magnetic separation and efficient attachment of surface through carbon-carbon bonds [32]. While the metal core is responsible for the high magnetic properties, the inert carbon shell provides a highly air and chemical stability. The nanobeads can be applied in the harsh conditions, such as low pH and high temperatures, without the problem of oxidation of the metal core. However, these ferromagnetic nanoparticles usually exhibit several drawbacks for biological applications namely their inferior dispersion stability (formation of agglomerates) in biological relevant solvents and their high unspecific binding tendency with proteins due to their hydrophobic surface. This creates an urgent need for a selective manetic sorbent which combines the advantageous properties of ferromagnetic (high saturation magnetization) and superparamagnetic (dispersion stability) nanoparticles. The covalent functionalization of their surface with a wide range of selective ligands allows achieving this goal. So, the ferromagnetic C/Co nanoparticles are covalently modified in order to enhance the dispersion stability as well as the antifouling properties [34].

Functionalization of magnetic particles with oligonucleotides

Bioconjugation can take place by means of adsorption (at the isoelectrical point of the oligonucleotide via electrostatic interaction), by covalent or noncovalent linkage between the surface of the nanoparticle and the oligonucleotide. The clear advantage of covalent linkages compared to physical adsorption is that the linkage prevents the competitive displacement of the adsorbed oligonucleotides by blood components, which occurs for adsorbed oligonucleotides [35].

Various types of magnetic particles have been used as solid supports for the attachment of oligonucleotides especially DNA aptamers. DNA fragments can be attached to magnetic particles either covalently (via carboxyl, hydroxyl or amino-groups on the bead's surface) or non-covalently streptavidin-biotin interactions (magnetic beads coated with streptavidin). In this review, several reactive *functional groups* such as carboxyl, streptavidin and maleimide are introduced for the surface coverage of magnetic particles. These fictionalized particles can easily be conjugated to oligonucleotides using the chemical reagents.

Carboxylic acid coated magnetic particles

Amine-terminated aptamers are coupled to carboxylated magnetic particles through carbodiimide reaction [36, 37]. The most common carbodiimide coupling strategy uses 1-Ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) as the coupling agents. The key advantage of EDC is that it provides one-step coupling. In addition, it involves no lengthy linker species, allowing the hydrodynamic radius of the NP to be minimized [38]. Unfortunately, EDC is indiscriminate; it can crosslink any two primary amines in the reaction. Using this reagent may yield a matrix of crosslinked oligos and micro-spheres. Another option is to bind biotin-labeled oligonucleotides to avidin/streptavidin-coated beads.

Streptavidin coated magnetic particles

The use of adapter molecules generally involves streptavidin and biotin for the formation of the complex. Streptavidin is a protein with four high-affinity binding sites for biotin. The biotin-streptavidin binding is very strong (K_D ~10^{-15} M) and resistant to high salt concentrations and urea [39]. Streptavidin coated magnetic particles are a solid-phase matrix for simple and efficient binding of biotinylated compounds [40]. Biotinylated aptamers attached to magnetic beads coated with streptavidin are often used for the purification of proteins [41].

Maleimide-activated magnetic particles

A maleimide may be used to conjugate primary amines to thiols [42]. In fact, maleimide activated magnetic particles are designed to conjugate ligands with thiol groups. The most commonly used maleimide-derived coupling reagent is sulfosuccinimidyl-4- (maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC). Maleimide coupling has been used to conjugate biomolecules

such as oligonucleotides and antibodies [43, 44].

Applications of oligonucleotide-based affinity magnetic separation

The combination of aptamer functionalized Fe_3O_4 NPs with upconversion NPs for ultrasensitive fluorescence detection of biological compounds

Upconversion nanoparticles (UCNPs), which can emit ultraviolet/visible (UV/Vis) light under near-infrared (NIR) excitation (typically 980 nm) are regarded as a new generation of nanoprobes because of their unique optical properties, including a virtually zero,auto-fluorescence background for the improved signal-to-noise ratio, narrow emission bandwidths and high resistance to photo-bleaching. These properties make UCNPs promising candidates as luminescent bioprobes in biomedicine and biotechnology [45, 46]. By combination of magnetic-field-assisted biochemical separation and concentration technology, novel composite materials of aptamer-functionalized magnetic NPs and UCNPs have been applied to the sensitive detection biological compounds.

Jiang et al. [47] developed a sensitive and stable bioassay coupled with affinity magnetic separation for the detection of Aβ oligomer (Aβo) as a potentially promising candidate biomarker for Alzheimer's disease diagnosis. The biotinylated Aβo aptamer was immobilized on avidin-conjugated Fe_3O_4 NPs, and its complementary DNA (cDNA) was linked to avidin-conjugated BaYF5:Yb,Er UCNPs, all through the biotin–avidin affinity reaction, in order to prepare the aptamer-Fe_3O_4 NPs and cDNA-UCNPs. Then the aptamer hybridized with cDNA to form the duplex structure on the surface of the Fe_3O_4 NPs/UCNPs nanoprobe, leading to the strong upconversion fluorescence signal. In the presence of Aβo, aptamer formed a defined quadruplex structure when binding to a target molecule, and caused the dissociation of some cDNA, liberating some UCNP-labeled cDNA, leading to a decreased upconversion fluorescent signal on the surface of Fe_3O_4 NPs. Then, after magnetic separation with an external magnet, highly specific and sensitive detection of Aβo was achieved in connection with the measurement of the upconversion fluorescent signal of the unreleased UCNPs on the surface of Fe_3O_4 NPs. In the present method, Aβo could be well separated with magnetic separation without requiring multiple pretreatment processes. Any autofluorescence originating from biomolecules possibly contained in solution could also be entirely avoided due to the use of infrared 980 nm laser excitation [48]. The decreased fluorescence intensity of UCNPs was related to the concentration of Aβo in the range of 0.2–15 nmol L^{-1} with a detection limit of 36 pmol L^{-1}. The developed method then was successfully applied to measure Aβo in artificial cerebrospinal fluid. Benefiting from the magnetic separation, the high sensitivity of UCNPs, as well as the selectivity and stability of the aptamer, the present strategy offered valuable information related to early diagnosis of Alzheimer's disease.

Duan et al. [49] reported a sensitive luminescent bioassay for the simultaneous detection of *Salmonella Typhimurium* and *Staphylococcus aureus* in water samples using aptamer-conjugated Fe_3O_4 NPs for both recognition and magnetic separation combined with UCNPs as highly sensitive dual-color labels. The bioassay system was fabricated by immobilizing aptamer 1 and aptamer 2 onto the surface of Fe_3O_4 NPs, which were employed to capture and separate S. Typhimurium and S. aureus. $NaY_{0.78}F_4$:$Yb_{0.2}$,$Tm_{0.02}$ UCNPs modified aptamer 1 and $NaY_{0.28}F_4$:$Yb_{0.70}$,$Er_{0.02}$ UCNPs modified aptamer 2 further were bond onto the captured bacteria surface to form sandwich-type complexes. Under optimal conditions, the correlation between the concentration of S. Typhimurium and the luminescent signal was found to be linear within the range of 10^1–10^5 cfu mL^{-1} (R^2=0.9964), and the signal was in the range of 10^1–10^5 cfu mL^{-1} (R^2=0.9936) for S. aureus. The limits of detection of the developed method were found to be 5 and 8 cfu mL^{-1} for S. Typhimurium and S. aureus, respectively.

Wu et al. [50] combined the highly specific recognition of aptamers with magnetic separation to develop an upconversion luminescent nanoprobe-based bioassay for the detection of trace ochratoxin A (OTA). They synthesized surface NH_2-group functionalized Fe_3O_4 NPs for the immobilization of avidin and further linked to a biotinylated aptamer. The aptamer functionalized magnetic NPs not only acted as recognition elements to recognize and capture the target substance, but could also favor the rapid separation and purification of the bound target and the fabrication of a simple assay in a homogeneous solution by using an external magnet. Under the optimal conditions, the decreased luminescent intensity was proportional to the concentration of OTA in the range of 1×10^{-13} gmL^{-1} to 1×10^{-9} gmL^{-1} with a detection limit of 1×10^{-13}

gmL^{-1}. The proposed method then was successfully applied to measure OTA in naturally contaminated maize samples.

Dai et al. [51] developed an easy and sensitive aptasensor for OTA based on magnetic separation and near infrared upconversion luminescence using an OTA specific aptamer and OTA aptamer cDNA, together with avidin-modified UCNPs and Fe_3O_4 NPs. The aptamer-UCNPs and cDNA-Fe_3O_4 NPs were hybridized to form a poly-network structure of Fe_3O_4 NP-UCNP nanocomposites. When the target OTA was introduced, the aptamer combined with the priority target and the cDNA-Fe_3O_4 NPs were replaced. The proposed method achieved a linear range between 0.01 and 100 ng mL^{-1}, with a detection limit as low as 0.005 ng mL^{-1}.

Liu et al. [52] described an aptamer based strategy for the magnetic separation of the antibiotic sulfadimethoxine. In this method, the UCNPs were deposited on the surface of magnetic NPs functionalized with the aptamer. In the presence of sulfadimethoxine, parts of the UCNPs dissociate from the surface of the magnetic NPs, and this results in decreased fluorescence intensity. The linearity was 1–9 ng mL^{-1} with a correlation coefficient of 0.9959, and the limit of detection of proposed method was calculated as 0.11 ng mL^{-1}.

Fang et al. [53] developed a sensitive aptamer based method for the magnetic bioseparation and quantification of oxytetracycline. The aptamer-modified magnetic NPs were employed as capture probes, and complementary cDNA-modified upconversion NPs were used as signal probes. Then, the probes were hybridized to form poly-network structure magnetic NPs-UCNPs signal probes. Finally, when the target was introduced, the aptamer combined with the priority target and the signal probe was replaced. The proposed method achieved a linear range between 0.05 and 100 ng mL^{-1}, and the limit of detection was as low as 0.036 ng mL^{-1}, benefiting largely from labeling with UCNPs, aptamer affinity and magnetic separation. Then, they successfully applied the method to measure OTC in milk samples.

Rapid multitarget magnetic separation through programmable DNA linker displacement

Probst et al. [54] have developed a simple yet robust multitarget affinity magnetic separation technology based on the clever concept of DNA strand-mediated displacement. The proposed method could quickly sort multiple targets in high yield and purity using selectively displaceable DNA linkers.

Aptamer coated magnetic beads for separation of biological compounds prior to their electrochemical determination

Wang et al. [55] developed an aptamer-based impedimetric bioassay using the microfluidic system for the sensitive and rapid detection of thrombin. Aptamer modified magnetic beads were used to capture and separate the target protein, and concentrated into a suitable volume. Then the complexes were injected into the microfluidic flow cell for impedance measurement. The results showed that the impedance signals have a good linearity with the concentrations of thrombin in a range from 0.1–10 nmol L^{-1} and the detection limit is 0.01 nmol L^{-1}.

Chen et al. [56] reported a homogeneous electrochemical bioassay for the detection of adenosine triphosphate (ATP) combining magnetic aptamer sequences for recognition and separation of target and a DNAzyme assisted cyclic signal amplification strategy. For this purpose, an efficient nanosorbent using dsDNA immobilized onto the surface of Au-SiO_2@Fe_3O_4 by S-Au interaction was fabricated prior to voltammetric determination of ATP. The proposed strategy showed an excellent performance such as low detection limit (0.5 pM), wide linear range (1 pM–10 nM and10 nM–1 μM) and analytical application in real samples.

Aptamer based magnetic separation of heavy metal ions before their determination by atomic spectrometry

The recent discovery of specific binding of Hg^{2+} to T-rich DNA, resulting in T-Hg^{2+}-T base pairs have led to the improvement of a number of methods for determination of mercury [57]. Our group [58] reported a simple and sensitive method for separation and preconcentration of trace amounts of Hg^{2+} from biological samples, based on magnetic beads coupled with thymine (T)-rich oligonucleotides. Mercury specific aptamers were conjugated on magnetic beads, and served as affinity probes to capture and separate trace amounts of the target. Then flow injection cold vapor atomic absorption spectrometry (FI CV AAS) is used for determination of mercury. The calibration curve for Hg^{2+} is linear from 0.12–87.5 μg L^{-1} with a detection limit of 0.05 μg L^{-1}, and the enrichment factor is about 280, which make it suitable for dilute solution analysis.

Our group [59] designed a selective and sensitive method for the determination of ultratrace contents of Pb^{2+} in biological samples, based on the guanine (G)-quadruplex formed by the aptamer with hairpin structure and Pb^{2+}. For this purpose, Pb^{2+} specific aptamer serving as an affinity probe to capture and separate trace amounts of the analyte, was covalently linked to Fe_3O_4/GO surface by using a suitable cross-linking agent. Then, the G-quadruplex complex was formed by the opening of the "neck- ring" of the hairpin structure of aptamer in the presence of Pb^{2+}. Inductively coupled plasma mass spectrometry (ICP-MS) was used for determination of Pb^{2+} in biological matrices. The calibration curve was linear over the range of 0.3–867.5 μg L^{-1} and an enrichment factor of 50 was obtained. The limit of detection was found to be 0.05 μg L^{-1}.

Wang et al. [60] described the colorimetric determination of Hg^{2+} in spiked river water based on the hybridization chain reaction of aptamer immobilized on the Fe_3O_4@Au NPs surface with its cDNA. The presence of Hg^{2+} inhibits this reaction and this enables less methylene blue to intercalate into the dsDNA structure. After magnetic separation of the Fe_3O_4@Au-aptamer carrying Hg^{2+}, the change in the absorbance of the residual MB solution is measured at 663 nm. The respective calibration plot is linear in the 1 to 300 nmol L^{-1} concentration range, with a 0.7 nmol L^{-1} detection limit.

Aptamer-based purification methods using magnetic beads

Lonne et al. [61] proposed a purification method based on the biological ligand-target interactions on the magnetic beads. For this purpose, an aptamer directed against the human Vascular Endothelial Growth Factor (VEGF) was selected as affinity ligand for establishing a purification platform for VEGF in small scale. Then, the aptamer was covalently immobilized on magnetic beads in a controlled orientation resulting in a functional active affinity matrix. The functionalized magnetic beads were utilized in protein binding experiments for characterization of VEGF binding.

Oktem et al. [62] reported the use of well characterized affinity magnetic beads for the immobilization of Taq-polymerase specific DNA-aptamer. DNA Taq-polymerase is a thermostable polymerase isolated from *Thermus aquaticus*, a bacterium that lives in hot springs and hydrothermal vents [63]. Taq-polymerase is an abbreviation of *Thermus Aquaticus* polymerase. It was demonstrated that the superparamagnetic poly(GMA-MMA-EGDMA) beads with immobilized Taq-polymerase specific DNA-aptamer allowed purification of the enzyme Taq-polymerase directly from recombinant bacterial crude extract. The purity of the eluted Taq-polymerase, as determined by HPLC, was 93% from the single-step purification protocol.

Mojsin et al. [64] reported a method for the rapid identification and purification of sequence specific DNA binding proteins based on magnetic separation. It has been shown that biotinylated DNA attached to streptavidin magnetic particles specifically binds the USF1 protein in the presence of competitor DNA. It has also been demonstrated that the protein could be successfully eluted from the beads, in high yield and with restored DNA binding activity. This procedure could be applied for the identification and purification of any high-affinity sequence-specific DNA binding protein with only minor modifications.

CONCLUSION

The rapidly growing field of biotechnology has created a critical need for simple, fast and highly sensitive processes for the separation and purification of biomolecules from biological solution. Since aptamers bind their targets with high affinity and specificity, they are promising alternative ligands in affinity separation. These synthetic oligonucleotides provide a much needed development in the field of affinity separation. The examples reported to date have demonstrated the promise oligonucleotide ligands offer as affordable, scalable and reliable alternatives to antibody-based affinity separation. As reviewed here, these synthetic affinity ligands have demonstrated success in a myriad of bioseparation and purification of proteins and DNA. However, magnetic separation in combination with adoption of oligonucleotide ligands, offer a complimentary approach to help alleviate speed, production and economic concerns in the downstream separation and purification of valuable biological compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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