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Review article

Aptamers as the 'capturing' agents in aptamer-based capture assays

Marimuthu Citartan [a,*], Ewe-Seng Ch'ng [a], Timofey S. Rozhdestvensky [b], Thean-Hock Tang [a,*]

[a] Advanced Medical & Dental Institute (AMDI), Universiti Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia
[b] Department of Medicine (TRAM), University Hospital of Muenster, Von-Esmarch-Str. 56, D-48149 Münster, Germany

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ABSTRACT

Aptamers are widely used as diagnostic agents due to their specificity and high binding affinity to their cognate target. Apart from diagnostic applications, aptamers can also be employed to capture their cognate target. Aptamer-based capture (AptaCapture) assays enable capture of the target by the aptamers followed by target purification. This review focuses on the immobilization strategies of aptamers, different target purification techniques, and applications of AptaCapture assays.

Contents

1. Introduction

Aptamers, which are single-stranded DNA (ssDNA) or RNA oligonucleotides that have high affinity and specificity to their target, are

* Corresponding authors.
E-mail addresses: citartan@usm.my (M. Citartan), tangth@usm.my (T.-H. Tang).

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generated via systematic evolution of ligands by exponential enrichment (SELEX) [1]. The recent upsurge in the number of aptamers isolated is due to their astounding features, which can rival those of antibodies [2]. Aptamers are a powerful tool for therapeutic and diagnostic applications. In diagnostics, aptamers are utilized as the sensor agents to capture their cognate targets [3–6]. The capture event is converted into a signal that can be measured both in a labelled [7] or label-free manner [8–10]. The signal corresponds to the presence or absence of the target. Specific purification of the aptamer-target complex is another potential application. In this regard, an aptamer can act as a capturing agent for its corresponding target, and the target captured by the aptamer then can be purified. This review focused on the applications of aptamers as capturing agents using aptamer-based capture (AptaCapture) assays. Immobilization of aptamers, different strategies for target purification, and the potential applications of AptaCapture assays are also discussed. Throughout this review, the word 'target' is used to represent the ligand that specifically interacts with the corresponding aptamer.

2. Aptamer immobilization for use in AptaCapture assays

The most important criterion of an AptaCapture assay is the immobilization of the aptamer on the surface platform without compromising its affinity for its cognate target. Several conjugation methods have been developed that differ in the biomolecular interaction between the terminal functional groups linked to the aptamer and surface platform-conjugated functional groups. The most widely employed technique for aptamer immobilization is biotin-streptavidin non-covalent binding [11,12]. Semi-covalent and non-covalent interactions formed by gold atom-thiol and amine-carboxyl pairs, respectively, are also used to immobilize aptamers. In most cases, aptamers are modified at the 5′- or 3′-end by these functional groups (biotin, thiol, or amino). These functional groups forge an interaction with their interacting pairs on the surface of the platform. Immobilization can also be achieved by the Watson–Crick base-pairing interaction of an additional sequence appended to the aptamer with its complementary sequence. This complementary sequence is conjugated with the functional groups at the 5′- or 3′-end. Importantly, the conjugation strategy chosen should avoid any steric interference or possible debilitation of the binding affinity during the incorporation of the functional groups to the aptamer. Wang et al. [13] recently reported that the immobilization orientations, immobilization methods, and spacers affect the accessibility of the aptamer to target living cells. Different surface proteins have different epitope presentations or accessibility to aptamers. Hence, only the most optimum aptamer orientation or immobilization can maximize its recognition of the target.

2.1. Streptavidin-biotin system

The non-covalent binding between streptavidin and biotin is one of the strongest interactions in nature. It has translational/rotational entropy (binding energy) of 4.5–6.0 kcal/mol [14]. The binding is rapid and stable over a wide range of pH values and temperatures [15]. The isoelectric point of streptavidin is 6.8–7.5, which minimizes non-specific adsorption within this pH range. Minimal non-specific binding with the surface platform is imperative for the maximum selective capture of a protein target from a complex mixture by the aptamers. In a study using aptamer-mediated cellular protein co-precipitation (co-aptoprecipitation), biotinylated DNA aptamers against human epidermal growth factor receptor (EGFR) and human insulin receptor (INSR) are immobilized on the surface of streptavidin-coated magnetic beads [16]. These aptamers specifically captured EGFR and INSR from a complex biological mixture. INSR was found to bind weakly with the streptavidin-coated magnetic beads, corroborating the low non-specific absorption associated with streptavidin.

In addition to specificity and high affinity, as streptavidin is homotetrameric in nature, each subunit is able to bind biotin with equal affinity. In the case of biotinylated aptamers, avidity associated with the homo-tetramericity increases the amount of target captured by the aptamers. As an example, Zhao et al. [17] generated a unique 3D network of aptamers that extended over micrometres of length into the solution and was able to efficiently capture the cognate target (Fig. 1). Analogous to the long tentacles of jellyfish that effectively catch and sting their prey, this 3D DNA network was created by rolling circle amplification from the circular template that contains the DNA aptamer generated against protein tyrosine kinase 7 (PTK7). The circular template was conjugated to biotinylated primers immobilized on the surface of the tetrameric avidin (analogue of streptavidin)-coated microfluidics device surface. This 3D DNA network system more efficiently captured the PTK7-expressing lymphoblast CCRF-CEMs compared to the monovalent aptamer and antibody. The biotin-streptavidin system should be avoided in AptaCapture assays that involve whole cells. As streptavidin contains the tripeptide sequence of Arg-Tyr-Asp (RYD), this can possibly promote its non-specific binding with whole cells [18]. Neutravidin, a deglycosylated avidin that is an analogue of streptavidin, is an option for whole cell capture, as it has no RYD motif.

Streptavidin can also serve as the target for aptamer isolation. The resulting anti-streptavidin aptamer acts as the anchorage for other proteins, aptamers, or RNA elements. Tahiri-Alaoui et al. [19] coupled the anti-streptavidin aptamer to the naturally occurring RNA elements CopT or CopA, which then were fused to the anti-CD4 aptamers to generate adaptamers. The CD4-streptavidin chimeric bi-functional aptamer was able to capture the target protein CD4.

2.2. Amine-carboxyl and amine-amine system

Another robust biomolecular interaction for aptamer immobilization is the covalent amine-carboxyl interaction [20–22]. An aptamer with primary amines such as 5′ Amino Modifier C6, Internal amino-C6-dT, or 3′ Amino Modifier appended to it can react with succinic anhydride in the presence of NHS (N-hydroxysuccinimide) and EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) to generate activated carboxyl groups [23]. Subsequently, the activated carboxyl group can interact with amine-terminated groups such as 3-aminopropyltriethoxysilane (APTES). APTES is usually immobilized on the surface of silanol-terminated silicon by the process known as silanization [24]. Inversely, APTES can be made carboxyl reactive via reaction with succinic anhydride and activation by NHS and EDC to readily react with amine-terminated aptamers [25]. For direct conjugation between the amine-conjugated primer and the amine of APTES, glutaraldehyde cross-linking can be used as the linker. The two aldehyde groups in glutaraldehyde interact with the primary amine to form imine bonds [26].

It is imperative to remove the unreacted aptamers from the immobilization surface, as carry-over of these unreacted aptamers into the reaction may result in non-conjugated aptamer-target formation. If this happens, the real amount of the target captured by the conjugated aptamer is reduced. During aptamer conjugation, protein contaminants should be avoided, as these 'uninvited' biomolecules can impart reactivity towards the amine or carboxyl-terminated surface.

2.3. Thiol-gold system

Conjugation of aptamers on a gold surface is actualized via semi-covalent-based interaction between the gold atom and thiol [27,28], which has a binding energy of 45 kcal/mol [29]. Functionalization with thiols is required prior to immobilization of aptamers on the surface of the gold. Functionalization can be achieved using the following sequential configurations: (1) a thiol (−SH) or disulphide (−SSR) terminus, (2) linker chains such as C6 [$(CH_2)_6$] or C11 [$(CH_2)_{11}$], (3) a spacer sequence such as poly A_{20}, and (4) the aptamer sequence. A spacer can