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APTAMERS AS SENSORS: REVIEW AND APPLICATION

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Abstract

Aptamers are nucleic acids that are ligand-binding and whose affinities and selectivities which rivals with those antibody. They are extracted from collections of 1012-1015 combinatorial oligonucleotides (DNA or RNA) chemically synthesised through a method known as in vitro collection. They have been modified not only as substitutes to antibody, but as special reagents in their own right for analytical applications. In general, through chemical or enzymatic synthesis, aptamers may be easily site-specifically modified to integrate specific reporters, linkers, or other moieties. Aptamer secondary structures may also be built to undergo analyte-dependent conformation modifications, which open up a wealth of potential signal transduction systems, regardless of whether the tracking modality is optical, electrochemical, or mass-based, in combination with the ability to precisely position chemical agents. Finally, since aptamers are nucleic acids, they are readily suited to amplification methods of sequence (and thus signal). Nevertheless, lack ofbasic understanding of their biochemistry or technological criteria, the application of aptamers may cause severe analytical complexities.

Keywords: *Aptamers, Detection, Optical Sensors, Reporters.*

I. INTRODUCTION

A. Aptamer Selection and Properties: -

Unlike typical nucleic acids, aptamers are single-stranded nucleic acids (RNA, DNA, and altered RNA or DNA) exhibiting special binding properties to their targets. These are chemically synthesised from 1012-1015 combinatorial oligonucleotide (DNA or RNA) libraries extracted through a technique known as in vitro collection. Very large colonies can be planted over numerous rounds of collection, and the few "fittest" species of nucleic acid can be extracted. Although it normally takes several weeks to months for the conventional in vitro selection process, recent system automation could provide a path from the target to the innovative reagent in just a few days[1].

A wide range of target molecules, comprising small organics, peptides, proteins, and even supramolecular complexes like viruses or cells, have been selected against various high-affinity and highly selective aptamers. The binding affinities of aptamers for different protein targets are extremely target based and range from the picomolar scale (1 x 10-12 M) to the high-nanomolar scale (1 x 10-7 M). The dissociation constant are greater (typically micromolar) when small organics are attacked, as would be anticipated considering the limited number of interactions that form. Interactions tend to be highly specific in either case, and aptamers may discriminate between even strongly linked substances like (a) an anti-theophylline aptamer, which, in turn, may discriminate against caffeine on the basis of characteristics of a single methyl group (6) or (b) an anti-kinase aptamer, which may differentiate among isozymes closely related. Aptamers have shown extraordinary potential in analytical applications outside these characteristics since they can be efficiently generated by chemical synthesis and can be easily changed with reporter molecules, linkers, and other functional groups. In order to produce allosteric enzymes or so-called aptazymes, aptamers may also be joined to nucleic acid enzymes (e.g., ribozymes and deoxyribozymes). Since aptazyme applications have recently been tested, some of their groundbreaking analytical applications are only briefly listed herein[2].

II. DISCUSSION

A. Optical Sensors: -

Incorporation of Single Reporters: Structural experiments have shown that after binding to their cognate ligands, aptamers often undergo major conformational shifts. Ligand binding can be transduced into a transition in the chemical environment of the fluorophore by inserting organic fluorophores into conformationally labile regions of aptamers, and thereby into a change in fluorescence characteristics such as strength and anisotropy. For instance, so-called signalling aptamers can be produced by labelling antiadenosine RNA and DNA aptamers with a fluorophore adjacent to the adenosine-binding site during synthesis. As a function of the ATP concentration (~30 μM for the DNA signalling aptamer and ~300 μM for the RNA signalling aptamer), the apparent Kd dependent on the dose-responsive fluorescence strength shift was much higher than that of the parental aptamers (~6 μM for the antiadenosine DNA aptamer and 6-8 μM for the antiadenosine RNA aptamer) (15, 16). As a common framework of reagents (aptamers or otherwise) that rely on conformational transduction for signalling, a decrease of binding affinity may be suggested[3].

B. Incorporation of Two Reporters: -

Strategy that focuses on more than one reporter have also proved feasible by allowing optical signal transduction based on fluorescence resonance energy transfer to be attempted (FRET). More and varied design technologies have been implemented as more and varying fluorophores and aptamer modifications have been commercially viable. The easiest format is to mark

aptamers with a quencher and either one or two fluorophores, making both techniques for quenching and "light-up[4]."

C. **Incorporation of Nanoparticle Reporters: -**

Generally, the fundamental transduction methods mentioned above are valid, and some reporters may be used to address problems usually associated with fluorescent dyes. In particular, quantum dots (QDs) and other nanoparticles provide a range of benefits for real-time control of biological processes over traditional fluorescent dyes, including greater photostability, greater efficient Stokes shifts, longer fluorescent lifetimes, and sharper emission bands than typical organic fluorophores. Furthermore, QDs all respond to the same wavelength of excitation but emit at different wavelengths, which helps with multiplexing. For the identification of cancer cells, bacterial spores (Bacillus thuringiensis), and proteins, Aptamer:QD conjugates were used (thrombin, with a LOD of 1 nM). Levy and colleagues, who coated the nanoparticle with several antithrombin aptamers hybridised to antisense strands containing quenchers, initially tailored QDs to aptamer beacon strategies. The quenchers were replaced in the presence of thrombin, and a 19-fold rise in fluorescence was observed in the QD beacon structure[5].

D. **Dye Displacement Assays: -**

It is not always feasible to manufacture highly functional biosensors, even given the different techniques mentioned for the design, engineering, and production of aptamer beacons, since the exact targeting sites and the conformational changes of the aptamers are normally not defined in atomic detail. In comparison, time-consuming and costly will be the covalent integration of reporters into the aptamer. Such considerations suggest an even easier way to modify aptamers to act as optical biosensors, as well as noncovalent binding and eventual analyte-dependent reporter relocation. Such relocation techniques can be treated as label-free approaches, because none of the assay reagents (neither aptamer nor target) needs any further adjustment prior to the assay and can prove to be extremely useful in drug and environmental testing for high-throughput screening. The displacement of fluorescent dyes, nevertheless, will lead to a signals decline, that is not ideal for the production of the assay[6].

E. **Electrochemical Detection: -**

Aptamer's analytical applications progress quickly towards electrochemical strategies, partly because electrochemical approaches are highly sensitive and partly because the instrumentation is simple, easily miniaturised and low-cost. As was the case for optical detection, aptamers have also been used in various electrochemical detection methods, including electrochemical impedance spectroscopy (EIS), ion-selective electrode potentiometry (ISEs), electrogenerated chemiluminescence (ECL), cyclic voltammetry, and amperometry. Aptamer capture of a target protein accompanied by EIS and chemical amplification (10 fM) and protein-induced oligonucleotide displacement coupled with ECLL was accomplished by detection in the femtomolar scale (1 fM). Interestingly, most of these strategies of identification have been restricted to DNA aptamers. Nevertheless, numerous researchers have recently documented the first instance of an RNA aptamer-based electrochemical sensor[7][8].

F. **Mass-Sensitive Detection: -**

The fact that aptamers can be easily synthesised and conjugated to surfaces has contributed to their use in many analytical methods, in particular mass-sensitive detection methods where identification is conducted on or at a surface, while conformational signal transduction is a major theme of this study. For instance, aptamers have been optimised for sensors such as surface plasmon resonance (SPR), surface acoustic wave (SAW), quartz crystal microbalance (QCM) and cantilever microchannel (measuring the degree of cantilever bending). These approaches usually do not need labelling to be included in the aim or other reagents, and are also known as label-free techniques. Since these approaches assess properties that are equal to the differential differences in density, comparatively large analytes (i.e. the same size as the aptamer itself or larger), but not tiny organic molecules or metabolites, are usually more relevant to them[9].

III. CONCLUSION

The sensitivities of aptamer sensors differ greatly as described in this study. These sensitivities are partially dependent on the aptamers' affinities for their targets; higher affinities typically generate lower LODs. In this respect, the usefulness of the aptamer sensor is expected to increase dramatically in the next generation of aptamers with a picomolar binding constant. While mechanisms of conformational transduction can be specifically designed for aptamers and have been shown to be adaptable to a wide variety of formats, the energy used to push the conformative transition comes from binding a ligand to the aptamer and must ultimately reduce the sensor's apparent affinity and sensitivity. The only exception to this principle is that quaternary structures are constructed on a target through multiple binding sites. In this situation, in the uncertainty of the target, the improbability of structure forming greatly decreases the context, making it possible to observe even very weak signals with assurance. While aptamers can be readily adapted to commonly used methods of nucleic acid amplification, in homogeneous assay formats these methods typically did not dramatically boost sensitivity. In particular, signal-amplification procedures usually did not lead to sensitivity improvements unless such immobilisation or washing steps were included in the assay. It is more likely that the sensitivity of the aptamer biosensor is impaired by the sensitivity of the analytical system used for its identification. Overall, while aptamer biosensors are likely to be increasingly used due to their ease of synthesis and reporter modular introduction possibilities, modern and increasingly responsive methodologies introduced by analytical chemists will eventually drive their sensor use. That is, of course, if the physiological nuances inherent in these reagents need to be attended to by analytical chemists.

IV. REFERENCES

[1] K. M. Song, S. Lee, and C. Ban, “Aptamers and their biological applications,” *Sensors*. 2012, doi: 10.3390/s120100612.

[2] E. J. Cho, J. W. Lee, and A. D. Ellington, “Applications of aptamers as sensors,” *Annu.*

Rev. Anal. Chem., 2009, doi: 10.1146/annurev.anchem.1.031207.112851.

[3] D. J. Patel *et al.*, "Structure, recognition and adaptive binding in RNA aptamer complexes," *Journal of Molecular Biology*. 1997, doi: 10.1006/jmbi.1997.1281.

[4] X. Fang, A. Sen, M. Vicens, and W. Tan, "Synthetic DNA aptamers to detect protein molecular variants in a high-throughput fluorescence quenching assay," *ChemBioChem*, 2003, doi: 10.1002/cbic.200300615.

[5] T. C. Chu *et al.*, "Labeling tumor cells with fluorescent nanocrystal-aptamer bioconjugates," 2006, doi: 10.1016/j.bios.2005.12.015.

[6] A. Norouzy, Z. Azizi, and W. M. Nau, "Indicator displacement assays inside live cells," *Angew. Chemie - Int. Ed.*, 2015, doi: 10.1002/anie.201407808.

[7] K. Ikebukuro, C. Kiyohara, and K. Sode, "Novel electrochemical sensor system for protein using the aptamers in sandwich manner," 2005, doi: 10.1016/j.bios.2004.09.002.

[8] A. E. Radi and C. K. O'Sullivan, "Aptamer conformational switch as sensitive electrochemical biosensor for potassium ion recognition," *Chem. Commun.*, 2006, doi: 10.1039/b606804a.

[9] Y. Li, J. L. Hye, and R. M. Corn, "Detection of protein biomarkers using RNA aptamer microarrays and enzymatically amplified surface plasmon resonance imaging," *Anal. Chem.*, 2007, doi: 10.1021/ac061849m.