Small molecule detection in solution *via* the size contraction response of aptamer functionalized nanoparticles

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ARTICLE INFO

ABSTRACT

We demonstrate a simple new sensor design that exploits aptamer functionalized nanoparticles (NPs) to transduce the signal of aptamer receptors binding to target small molecules. An aptamer capable of binding to our target 17β-estradiol (E2) was isolated by SELEX with dissociation constant of 50 nM and tethered to the surface of carboxylated polystyrene NPs. Upon exposing the aptamer functionalized NPs to E2 in buffered water, we use dynamic light scattering (DLS) and resistive pulse sensing (TRPS) to observe a distinct reduction of the conjugated particle size and a less negative zeta potential, which can be correlated to the E2 concentration in the lower nanomolar range. The sensor showed similar affinity towards other hormones of the E2 steroidal family and excellent discrimination against potential non-steroidal interfering agents. The simplicity of the sensing scheme makes it readily applicable to other low molecular weight targets, as we further demonstrate using a known adenosine aptamer. In addition to sensing, our method shows potential to guide the synthetic evolution of aptamers with better binding affinity and specificity.

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1. Introduction

Single stranded (ss) DNA aptamers, discovered in 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990), have demonstrated considerable promise for sensing applications owing to the combination of several important properties. Firstly, the synthetic evolution process through which optimal ssDNA sequences are refined can be directed towards sensitive (femtomolar concentrations) and selective detection of a broad spectrum of analytes, including proteins, small molecules and even toxins. Secondly, once an aptamer sequence is identified, it can easily be synthesized and augmented with further functionality or coupled to other materials (Lee et al., 2010; Song et al., 2008; Stoltenburg et al., 2007). Moreover, the relatively small size of aptamers (generally on the order of 20–100 nucleotides long, corresponding to fully extended lengths of 6.8–34 nm) compared with other recognition elements such as antibodies opens up the possibility of simple new physical means of signal transduction (Keefe et al., 2010; Lee et al., 2010, 2008).

The ability of aptamers to recognize small molecular targets including hormones and narcotics is linked to the aptamers' propensity to adopt a folded three-dimensional conformation that provides a specific binding pocket for the target (McKeague and Derosa, 2012; Wang et al., 2010). Signals can then be transduced from the target-induced conformational switching *via* mechanisms that are not readily available to conventional antibody-based receptors or for aptamers targeting macromolecules that are larger than the aptamer. For example, when pairs of fluorescent dyes are attached to different sections of the aptamer, binding the target may bring the dyes into close enough proximity to result in detection *via* fluorescent resonant energy transfer (Stojanovic et al., 2001). Alternatively, a redox probe can be attached to the distal end of an aptamer immobilized on an electrode surface such that binding the target brings the redox probe within electron transfer distance of the electrode and a current is measured (Tong et al., 2011). While these methods demonstrate the ability to detect binding induced changes in aptamer conformation with high sensitivity, their successful implementation in a given system requires detailed knowledge of specific aptamer conformations and target interaction sites, as well as more design complexity, in order to engineer strong coupling between the binding event and signal from the attached probes.

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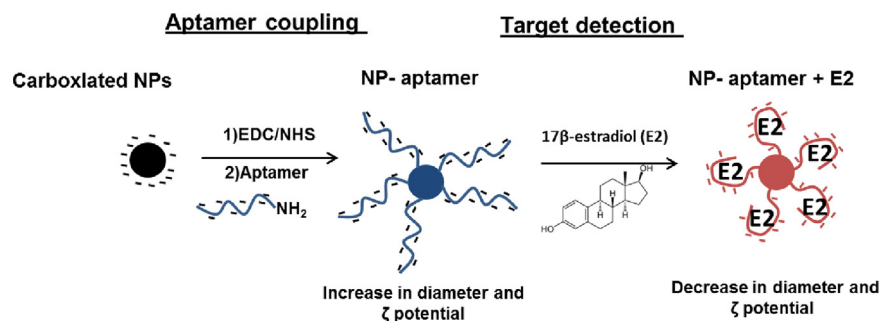
Scheme 1. Overview schematic of a NP coupling to amino-terminated aptamers and the formation of a NP–aptamer conjugate, followed by the detection of E2 molecules and formation of an aptamer–E2 complex.

Herein, we explore a simple alternative means to detect the binding of a small molecule in solution *via* measuring the contraction in aptamer size that generally accompanies the binding of a small molecule, but without regard to the specific conformation of an aptamer–target complex. The key to our approach, depicted in Scheme 1, is to tether the aptamers to the surface of NPs and resolve the changing size of aptamer functionalized particles when target molecules induce a more tightly-folded aptamer conformation on the particle surface. The success of our strategy is ensured by the availability of functionalizable NPs of a suitable size and excellent monodispersity combined with the modern solution-based particle sizing techniques to resolve diameters with better resolution than the binding induced changes in average aptamer size.

We demonstrate our technique with an aptamer developed to target the hormone 17β-estradiol (E2), as well as showing its generality *via* a known aptamer for adenosine. The adverse health effects arising from endocrine disrupting compounds like E2 accumulating in the environment (Diamanti-Kandarakis et al., 2009) highlights the need to develop sensors that are more cost- and time-effective than the presently used chromatographic and mass spectrometric analytical techniques (Auriol et al., 2006; Pacáková et al., 2009). We are able to detect the presence of ~5 nM E2 in buffered water by measuring changes in particle sizes *via* both DLS and TRPS – a recently developed particle-by-particle sizing technique (Kozak et al., 2012; Platt et al., 2012; Willmott and Moore, 2008; Willmott and Parry, 2011). We also observe a clear correlation between NP–aptamer size contraction and surface potential due to the redistribution of charge when the polyanionic aptamer folds around its target, thus extending the range of simple means to transduce binding signals in our system. The sensitivity of our technique will be improved with the refinement of aptamer–target binding affinities but, compared with other small molecule aptasensors, has few other design constraints associated with engineering signal transduction.

2. Materials and methods

2.1. Chemicals and reagents

2-(N-morpholino)ethanesulfonic acid (MES), tris(hydroxy-methyl) amino methane (Tris–HCl), N-(3-dimethylaminopropyl) N-ethylcarbodiimide hydrochloride (EDC), N hydroxy-succinimide (NHS), NaCl, MgCl$_2$, CaCl$_2$, KCl, polyoxyethylene (9) nonylpheny-lether (IGEPAL®, non-ionic detergent), streptavidin–peroxidase conjugate, luminal, p-coumaric acid, 17β-estradiol (E2), progester-one, testosterone, Bis (4-hydroxyphenyl) methane (BPF), bisphenol-A (BPA) and adenosine were purchased from Sigma-Aldrich. The E2 aptamer was isolated by SELEX process from a random ssDNA library composed of approximately 7.2×10^{14} DNA molecules. The random ssDNA library, biotin-aptamer and primers were purchased

from Life Sciences, Australia. The amino terminated E2 and adeno-sine (previously reported by (Kim et al., 2009)) aptamers were synthesized and PAGE purified by Sigma-Aldrich. For the fluores-cence experiment to determine the aptamer density on NPs, a closely related ssDNA sequence of 76-mers previously used by (Kim et al., 2007; Yildirim et al., 2012) for the detection of E2, was used after modification with 5'-amino (CH$_2$)$_6$ and 3'-Cy5.5 groups (F-aptamer) by Eurofins Genomics India Pvt. Ltd. All aptamers were rehydrated in Milli-Q water and kept at −5 °C before use (unless stated). Blotto blocking solution (5% solution of non-fat powdered milk in Tris-buffered saline) was purchased from Life Sciences. GBX developing solution, X-ray cassettes, fixing solution and scientific imaging X-ray films were purchased from Kodak, Rochester, New Jersey, USA. Hydrogen peroxide was purchased from Scharlau Chemie, Adelaide, Australia. Nitrocellulose membranes were purchased from Amersham GE Biosciences, Little Chalfont, UK. Standard 217 nm NPs (Bangs Laboratories, Indiana, USA) were purchased unmodified from Izon Science (Christchurch, New Zealand). The NPs are nominally supplied with a mode size of 217 nm and a solid content of 10.1 wt%, corresponding to a concentration of 1.763×10^{14} particles g^{-1} (1.79×10^{17} particles mL^{-1}). The carboxyl group concentration in sur-face titration is 86 μeq g^{-1}. All other chemicals used were of analytical grade. Milli-Q water was used for all solution preparation.

2.1.1. Binding washing buffer (BWB)

2 mM Tris–HCl at pH 7.5 (containing 10 mM NaCl, 0.5 mM KCl, 0.2 mM MgCl$_2$, 0.1 mM CaCl$_2$ and 5% ethanol) was prepared ($I = 0.11$ mM, pH 7.5, resistivity 0.848 Ω m, viscosity 0.891 mPa s, relative permittivity 78.5). 0.1% IGEPAL® was added for TRPS experiments for wetting purposes.

2.1.2. Hybridization buffer

0.1 M MES at pH 6.2 was prepared ($I = 0.1$ mM, viscosity 1.00 mPa s, relative permittivity 78.5). EDC/NHS solution was prepared in MES at concentrations of 0.01 M, and this solution was kept at 4 °C for a week and then discharged. Stock 100 μM ethanol solutions of E2 and the interfering agents were prepared, and the detected concentrations were prepared in BWB, with the ethanol quantity held constant at 5%. NPs were suspended in MES buffer at a concentration of 5.2×10^{10} particles mL^{-1}.

2.2. NP functionalization

E2 aptamer of the following sequence was functionalized to NPs: 5'-NH$_2$(CH$_2$)$_6$-ATACGAGCTTGTTCAATACGAAGGGATGCCGTT-TGGGCCCAAGTTCGGCATAGTGTGGTGATAGTAAGAGCAATC-3'.

2.2.1. NP functionalization

400 μL of carboxylated NPs (5.2×10^{10} particles mL^{-1}) in MES were sonicated for 10 min, following which 200 nmol of EDC and