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Biomolecular detection at ssDNA-conjugated nanoparticles by nano-impact electrochemistry

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ABSTRACT

We describe the use of ssDNA functionalized silver nanoparticle (AgNP) probes for quantitative investigation of biorecognition and real time detection of biomolecular targets using nano-impact electrochemistry. The method is based on measurements of the individual collision events between ssDNA aptamer-functionalized AgNPs and a carbon fiber miroelectrode (CFME). Specific binding events of target analyte induced collision frequency changes enabling ultrasensitive detection of the aptamer target in a single step. These changes are assigned to the surface coverage of the NP by the ssDNA aptamers and subsequent conformational changes of the aptamer probe which affect the electron transfer between the NP and the electrode surface. The method enables sensitive and selective detection of ochratoxin A (OTA), chosen here as a model target, with a limit of detection of 0.05 nM and a relative standard deviation of 4.9%. The study provides a means of characterizing bioconjugation of AgNPs with aptamers and assessing biomolecular recognition events with high sensitivity and without the use of exogenous reagents or enzyme amplification steps. This methodology can be broadly applicable to other bioconjugated systems, biosensing and related bioanalytical applications.

1. Introduction

The convergence of nanotechnology and biotechnology has led to the development of hybrid bioanalytical sensing platforms with increased sensitivity, stability and potential for miniaturization and portability in analysis (Katz and Willner, 2004). Hybrid nanomaterials that incorporate the selectivity and recognition functions of bioreceptor molecules with the unique size-dependent properties of NPs have proved to be of substantial benefit to the development of bioanalytical assays for clinical diagnosis, biosensing, environmental and food monitoring. A variety of conjugation techniques are available to attach biomolecules to NPs (Ravindran et al., 2013; Sperling and Parak, 2010). While widely used, control and characterization of bioconjugation and evaluation of the functionality of the immobilized biomolecule with adequate sensitivity remain a challenge. Attachment of biomolecules can affect the structure of the biomolecule (Vertegel et al., 2004) while excessive coverage can reduce its activity due to steric crowding (Demers et al., 2000). The ability to investigate biomolecular recognition events at the surface of single particles is important for the rational design of molecular recognition probes for a variety of applications including therapeutics, cell tracking, drug delivery systems, biosensing and for the development of sensitive bioanalytical assays.

Among bioconjugated NPs, aptamer functionalized NPs that use artificial single stranded oligonucleotides have gained popularity in recent years due to their high stability and ability to recognize and bind to a variety of targets with high specificity and selectivity (McConnell et al., 2014). When bound to a target, aptamers undergo significant conformational changes (Fan et al., 2003). The folding/unfolding of aptamers is essential for their recognition ability. Commonly used methods to characterize NP-aptamer bioconjugates include UV–vis and fluorescence spectroscopy (Yu and Irudayaraj, 2006) that rely on bulk measurements of the conjugate system. Achieving accurate quantitative data on the binding using these methods is difficult because of relatively small changes in the optical spectra, background interferences and spectral overlap. Combining UV–vis spectroscopy and chemometrics by introduction of multivariate curve resolution enhanced the resolving power of traditional UV–vis spectroscopy (Wang and Ni, 2014). Incorporating a fluorescent label at the terminus of the DNA sequence enabled quantitation by fluorescence spectroscopy through displacement of the surface-bound strands (Demers et al., 2000). Enzyme hydrolysis of fluorescent-labeled biomolecules, stepwise photobleaching of organic fluorophores, single particle fluorescence resonance energy transfer (spFRET), adsorption isotherms, and gel electrophoresis have also been used to assess the coupling of biomolecules to NPs (Pons et al., 2006). In this work,

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we describe a novel single step and sensitive electrochemical approach for the characterization of aptamer conjugated NPs and detection of biomolecular targets using nano-impact electrochemistry.

The rapidly growing field of nano-impact electrochemistry involving measurement of single particle collision events with an electrode surface has made a significant impact on the study of NP systems. Nano-impact electrochemistry has demonstrated promise for the detection and characterization of a variety of NPs including metals, metal oxides and organic particles (Cheng et al., 2013; Sardesai et al., 2013; Xiao and Bard, 2007), and could be of use for the characterization of NPs in a variety of environments (Ozel et al., 2014; Sardesai et al., 2013). Starting from the pioneering work of Bard group (Xiao and Bard, 2007), several groups including Stevenson (Dasari et al., 2013), Crooks (Alligrant et al., 2015), Compton (Cheng and Compton, 2014; Rees et al., 2012; Stuart et al., 2012), Zhang (Guo et al., 2014), Alpuche-Aviles (Fernando et al., 2013), and a few others have all made significant contributions to the field of nano-impact collision electrochemistry. Previous researches demonstrated the ability of the method to evaluate the catalytic properties, size distribution, concentration, aggregation degree and adsorption processes at NPs (Cheng and Compton, 2014; Rees et al., 2011; Zhou et al., 2010). More recently, additional capabilities have been demonstrated to measure *E. Coli* bacteria decorated with AgNPs (Sepunaru et al., 2015), NPs tagged with electroactive molecules (Zhou et al., 2012), analysis of DNA hybridization by indirect measurement of hydrazine oxidation (Kwon and Bard, 2012) and for detection of viruses by antibodies modified polystyrene beads and single-protein and DNA macromolecule (Dick et al., 2015). The group of Crooks (Alligrant et al., 2015; Alligrant et al., 2013) has recently reported on the electrocatalytic amplification on single collisions of PtNPs using DNA-modified surfaces. Despite significant progress on the development of this method, studies to investigate the capability of this method in the bioanalytical and biosensing field for detection of biomolecular targets are limited.

Here we investigate the use of NP collision electrochemistry as a detection technique for characterizing bioreceptor NP conjugates and quantitatively measuring target analytes using aptamer-modified AgNPs. As a proof of concept, we developed the method for detection of OTA. OTA is one of the most-abundant food-contaminating mycotoxins produced by fungi species such as aspergillus and penicillium. OTA is classified as a potential carcinogen (group 2B) for humans (Ha, 2015). Several types of electrochemical aptamer-based bioassays have been developed using target-induced structure switching mode by probing the electron-transfer at aptamer modified electrodes in various configurations (Bulbul et al., 2015a, 2015b). Most of these methods involve sensitive enzyme labels, soluble redox mediators, extensive electrode modification or multistep procedures to achieve the required detection limits for practical applications. As opposed to previous studies, here we describe a single step procedure that employs a bare CFME and AgNPs modified with aptamers and uses NP collision electrochemistry to characterize the transient current generated upon the individual impact of aptamer-modified AgNPs with the electrode. Changes in the current response from collisions of conjugated aptamer-AgNPs enable real time quantitative detection of the aptamer target with high sensitivity. The method can be particularly useful in fundamental studies of binding mechanisms of aptamer-NP conjugates, and in biosensing/bioanalytical applications that require recognition and ultrasensitive detection of biomolecular targets.

2. Materials and methods

2.1. Reagents and chemicals

5′-amino-modified aptamer strands were provided by Eurogentec (North America Inc.). The sequences for the given strand is (5′ NH_2-GAT-CGG-GTG-TGG-GTG-GCG-TAA-AGG-GAG-CAT-CGG-ACA 3′). Phosphate buffer (PB) solution, 0.1 M, pH 7.4 was prepared from sodium phosphate (monobasic and dibasic) (Fisher Sci.). The binding buffer (BB) was prepared from 1 mM $MgCl_2$, 140 mM NaCl, 2.7 mM KCl. OTA (from *A. ochraceous*) and Ochratoxin B (OTB) were first dissolved in ethanol and then diluted in binding buffer. OTA and OTB were supplied by Sigma-Aldrich. Carbon fibers were purchased from WPI (World Precision Instruments Inc.). Silver conductive epoxy was obtained from MG Chemicals. Non-conductive epoxy was purchased from Devcon. Distilled, deionized water (Millipore, Direct-Q System) with a resistivity of 18.2 Ω cm was used. Citrate capped silver nanoparticles (AgNPs) were generously provided by Dr. Dan Goia, Clarkson University.

2.2. Instrumentation

Electrochemical experiments were performed using a CH Instrument analyzer (CH Instruments Inc.) (Austin, USA). All experiments were carried out using a conventional electrochemical cell with cylindrical carbon microelectrodes as the working electrode, Ag/AgCl/3 M KCl as reference electrode and a platinum wire as counter electrode. All potentials were referred to the Ag/AgCl reference. UV–vis studies were performed with a Shimadzu PC-2401 spectrophotometer (Kyoto, Japan). Transmission Electron Microscopy (TEM) experiments were performed using a JEOL 2010 High Resolution Scanning Transmission Electron Microscope (HRTEM) (Peabody, USA). Samples were dried before measurements. Particle sizing and zeta potential measurements were carried out using a Brookhaven Instruments Zeta PALS analyzer (Holtsville, USA).

2.3. Preparation of cylindrical carbon fiber microelectrodes

Single CFME with a diameter of ~2.5 μm were fabricated from individual carbon fibers from WPI. A single carbon fiber was glued to a copper wire with a conductive silver epoxy paste and aspirated into a pulled glass capillary tube. The upper end of the capillary tube was sealed with a non-conductive epoxy resin and cured at 100 °C for 10 min. The capillary tube with the connecting wire was pulled using a Narishige PP-83 microelectrode puller. The length of the fiber extending from glass was fixed to ~1 mm from the glass seal using a scalpel. Microelectrodes were immersed in isopropanol for 10 min to clean the fibers and air dried. Before experiments the electrodes were characterized by cyclic voltammetry (CV) using the potassium ferricyanide/ferrocyanide redox couple to determine the surface area and ensure batch-to-batch reproduciblility.

2.4. Preparation of AgNP aptasensor

Prior to modification, the aptamer solution was heated at 95 °C for 5 min and subsequently left at room temperature for 3 min. Citrate capped AgNPs were used as support for the immobilization of the 5′ amino aptamer through the amino group. The immobilization protocol consisted of the following steps: (1) 10 mg AgNP were dispersed in 1 ml PB and sonicated. (2) The dispersion was centrifuged and 1 ml 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) at a concentration of 15 mg/ml was added to the dispersion to activate the carboxylic acid groups of the citrate capped NPs. (3) The mixture was incubated using a shaker for 3 h