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Colorimetric detection of controlled assembly and disassembly of aptamers on unmodified gold nanoparticles

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ABSTRACT

Aptamers are nucleic acid ligands that are generated artificially by *in vitro* selection and behave similar to antibodies. The development of aptamer-based sensing systems or strategies has been in vogue for the past few decades, because aptamers are smaller in size, stable, cheaper and undergo easier modifications. Owing to these advantages, several facile aptamer-based colorimetric strategies have been created by controlling the assembly and disassembly of aptamers on unmodified gold nanoparticle probes. As these kinds of assay systems are rapid and can be visualized unaided by instruments, they have recently become an attractive method of choice. The formation of purple-colored aggregates (attraction) from the red dispersed (repulsion) state of GNPs in the presence of mono- or divalent ions is the key principle behind this assay. Due to its simplicity and versatility, this assay can be an alternative to existing diagnostic assays. Here, we have investigated the critical elements involved in colorimetric assays, and have screened different proteins and small ligands to evaluate biofouling on GNPs.

Contents

1. Introduction

Nanomaterials can facilitate signal transduction with electroactive tags for sensing and imaging purposes. The benefits obtained from nanotechnology mainly depend on specific tailored materials designed with essential structures at the nanoscale level to achieve a specific goal, thus greatly extending the range of applications, including diagnosis. Among the several nanomaterials that have been fabricated, the gold nanoparticle (GNP) is an ideal material that is widely used in the development of sensors, owing to its unique characteristics, such as easy water dispersal, compatibility with surface functionalization, biological non-reactivity, and ability to be tailored with uniform and different nano-sizes (Lim et al., 2011; Upadhyayula, 2012; Guirgis et al., 2012). GNPs adsorb in the

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visible light spectrum around 520 nm (green light) due to the excitation of plasmons in the particle and this wavelength can be adapted to several optical sensors (Nagel et al., 2011; Tinguely et al., 2011; Gopinath et al., 2012a,b,2013). In the past, several attractive gold-based sensing surfaces have emerged, and the applications of these surfaces have expanded to sensor development in conjunction with various fields (Baldrich et al., 2004; Varma et al., 2004; Guo, 2005; Gronewold et al., 2005; Odenthal and Gooding, 2007; Peng et al., 2007; Wang et al., 2008; Gopinath et al., 2008a; Marquette and Blum, 2008; Song et al., 2008; Giljohann et al., 2010; Iliuk et al., 2011; Zanoli et al., 2012).

Beneficial sensing systems have several characteristics, including ease of use, low cost, rapid process, and results that are easily understandable without prior knowledge and distinguishable by the naked eye. Several sensing systems that exhibit these characteristics have been proposed and commercially established, including color-based sensing on membrane filters (such as immunochromatography tests) or solution-based colorimetric assays (Pavlov et al., 2004; Huang et al., 2005; Liu and Lu, 2006; Wang et al., 2007; Wei et al., 2007; Chen et al., 2008; Zhao et al., 2008a; Li et al., 2009; Lau et al., 2010; Kim et al., 2010; Song et al., 2011; Chávez et al., 2012; Selvakumar and Thakur, 2012). However, the compatibility of the biomolecules used as ligands or analytes must be ascertained. Considering these basic parameters of ideal nanomaterials, i.e., visual interpretation of assay results, and biocompatibility, GNP-based colorimetric assays might be the suitable candidates. GNP-based colorimetric assays can be efficient biosensors, as the molecular recognition event can be transduced visually, obviating the need for any special equipment (Zhao et al., 2008a; Kim and Jurng, 2011). The involvement of interparticle plasmon coupling or local refractive index change-induced plasmon band shift makes this colorimetric assay facile. GNPs are capable of adsorbing small oligonucleotides due to their propensity for electrostatic attractions, hydrophobic absorption, and covalent binding (Zhao et al., 2008a; Lou et al., 2012). Under the category of small oligonucleotides, aptamers have been developed rapidly for use in diagnosis, drugs, molecular validation, etc. (Gopinath, 2011). The features of aptamers including their variety of structural conformations, and their selectivity and specificity, yield excellent matches with target molecules and are ideal for aptasensor development.

Aptamers are artificially evolved molecules against specific targets, from small molecules to whole cells, that are generated by the separation and amplification processes using randomized RNA or DNA libraries. This strategy involves an in vitro selection process, called 'systematic evolution of ligands by exponential enrichment' (SELEX). This selection process mimics the natural selection of aptamers as reported in the case of riboswitches (Breaker, 2011). A typical aptamer generation process starts with about 10^{14} molecules, under stringent conditions. Only high-affinity molecules are retained through successive selection cycles (Fig. 1). The selected aptamers in the past have possessed significant ($>$ 10,000-fold) abilities to discriminate between closely related molecules (Jenison et al., 1994; Geiger et al., 1996). The ability of aptamers to discriminate between molecules has led to the development of high-performance sensing systems. Several functionalization chemistries or modification procedures are available for sensing plates or on aptamers to form the appropriate anchorage and create aptasensors. However, these approaches may have limitations with lower-affinity interactions due to structural changes upon aptamer modification, high cost, and low yield of modified products. The strategy of spontaneous adsorption becomes an easy way to resolve these issues. As demonstrated previously, the GNP-based colorimetric assay is a popular strategy for aptamer–ligand interactions, and does not involve modifying the analyst or ligands (Pavlov et al., 2004; Wei et al., 2007; Wang et al., 2007; Kim et al., 2010; Selvakumar and Thakur, 2012). In the present overview, the colorimetric detection of the controlled assembly and disassembly of GNP-based aptamer–ligand interactions has been described. Further, the suitability of these assays for biomolecular interactions are validated by testing their bio-fouling and non-biofouling characteristics.

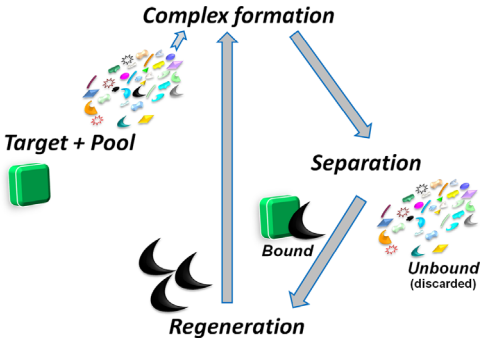

Fig. 1. Schematic representation of the process of 'systematic evolution of ligands by exponential enrichment (SELEX)'. This process involves the selection of high-affinity molecules from a randomized library of molecules by separation and amplification processes, under stringent conditions. Only high affinity molecules are retained with successive selection cycles. After a few to several rounds of selection cycles, molecules with higher affinities are cloned and sequenced to identify the selected candidates.

2. Factors influence GNP-based colorimetric assays

Colorimetry is a solution-based assay that is used to determine the concentration of colored compounds. A colorimeter or standard spectrophotometer can be used to estimate the concentration of materials in the solution by measuring its absorbance at a suitable wavelength. In this assay, the length of the light path through the solutions is measured by absorbance, with the units of optical density. The color or wavelength of the filter chosen for the colorimeter is the critical one, as the wavelength of light that is transmitted by the colorimeter has to be same as that absorbed by the substance being measured. With these basic parameters, several GNP-based colorimetric assays for aptamer–ligand interactions have been developed; these assays also depend on several other parameters as described below.

2.1. Effects of mono- and divalent ions

This assay involves the strategy of using balanced interparticle attractive or interparticle repulsive forces to cause aggregation or dispersion, respectively (due to gain or loss of surface charges); van der Waals attractive forces on the surface also cause aggregation (Zhao et al., 2008a). These two states are determined by the presence or absence of ions in the gold colloidal solutions. The as-received gold colloidal solution exhibits strong absorbance of visible light at a wavelength of 520 nm, due to the excitation of plasmons (Fig. 2a) (Nagel et al., 2011; Tinguely et al., 2011; Gopinath et al., 2012b, 2013). Due to the above mechanism, in the presence of salts, GNPs can change color from red to purple (Fig. 2b). The addition of NaCl or other salts caps the repulsion among unmodified negatively charged GNPs, inducing the aggregation of these particles and resulting in a purple or blue solution. To study the dose dependence of these changes on monovalent ions (NaCl), we titrated different concentrations of NaCl from 15 to 120 mM with serial 50% dilutions (15, 30, 60, and 120 mM). As shown in Fig. 2b, with increasing concentrations of NaCl, there were gradual changes in the color of the GNPs from red to purple. At the 15 mM concentration, the color of the solution was reddish-