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High-sensitivity detection of ATP using a localized surface plasmon resonance (LSPR) sensor and split aptamers

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ABSTRACT

A highly sensitive localized surface plasmon resonance (LSPR) aptasensor for detection of adenosine triphosphate (ATP) has been developed. The sensor utilizes two split ATP aptamers, one (receptor fragment) being covalently attached to the surface of a gold nanorod (GNR) and the other labeled with a random DNA sequence and TAMRA dye (probe fragment). In the presence of both ATP and the probe fragment, a significant shift takes place in the wavelength of the LSPR band. This phenomenon is a consequence of the fact that the split fragments assemble into an intact folded structure in the presence of ATP, which brings about a decrease in the distance between the GNR surface and TAMRA dye and an associated LSPR wavelength. By using this sensor system, concentrations of ATP in the range of 10 pM–10 μM can be determined. In addition, by taking advantage of its denaturation properties, the LSPR aptasensor can be reused by simply subjecting it to quadruple salt-addition/2 M NaCl washing steps. That the new method is applicable to biological systems was demonstrated by its use to measure ATP concentrations in *E. coli* and, thus to determine cell concentrations as low as 1.0×10^3 CFU.

1. Introduction

Adenosine triphosphate (ATP) is one of the most important small molecule signaling agents. This triphosphate plays a central role not only in storage of metabolic energy, but also in signal transduction (Buzzi et al., 2010; Eliston et al., 1998; 2010; Jones, 1972). Intracellular ATP levels have been used as indicators for cell viability as well as many diseases such as hypoxia, hypoglycemia, ischemia, Parkinson's disease, and some malignant tumors (Dale and Frenguelli, 2009; Evans et al., 2004; Kahlin et al., 2014; Keane et al., 2011; Ito et al., 2014). In addition, in food and agricultural chemistry, and in the health sciences, measurements of ATP concentrations in real samples are required for bacteria detection in order to prevent food-borne illnesses (Jin et al., 2015). Therefore, the availability of reliable and sensitive methods for specific detection of ATP is important for biochemical, clinical, and food applications.

A variety of methods have been designed to detect ATP including enzymatic cycling (Ishida et al., 2008), protein regulating (Gray et al., 2014), and aptamer-based strategies (Wang et al., 2015; Xie et al., 2014). As a consequence of the increasing applications of aptamers as new recognition elements, attention has been given to aptamer-based approaches for selective detection of ATP. Sensors of this type take advantage of the high binding affinities and specificities as well as structural switching properties of aptamers in combination with amplification strategies (Kim et al., 2008a; Liu et al., 2014). Although various optical and electrochemical ATP aptasensors have been developed, all suffer from insufficient sensitivities. As a result, simple, versatile and highly sensitive methods are required that enable amplification of signals associated with sensing ATP.

A LSPR, which occurs in unique nano-sized metal particles such as gold and silver, is a consequence of isolated electronic oscillation in metal nanoparticles. The LSPR phenomenon is characterized by the presence of a specific absorption band in the visible or near-infrared wavelength region, the position of which is influenced by the dielectric environment near the nanoparticle surface (Haes et al., 2006; Kim et al., 2008b). Molecular interactions at the surfaces of the nanostructures lead to local refractive index (RI) changes and an associated red-shift of the LSPR band. This unique

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optical property is the basis for employing nanostructure-based sensors to investigate binding interactions of biochemicals and chemicals in a label-free manner (Lee et al., 2013; Mayer et al., 2008; Park et al., 2009; Zhou et al., 2008). Many LSPR biosensors that rely on the use of metal nanoparticles have been developed for detection of biomolecules, such as DNA, proteins and cells (Liu et al., 2013, 2010; Yoo et al., 2015). In the new sensing system developed in this effort, a LSPR-based method was employed for ATP detection and an aptamer was used to recognize ATP.

However, most often LSPR-based detection of small molecules such as ATP typically lacks sufficient sensitivity owing to the fact that only small RI changes occur when compounds having low molecular weights and/or low densities are present in the sensing volume of the nanoparticle. The sensitivity of techniques based on LSPR is dependent on the distance between the analyte and the surface of the nanoparticle and the associated RI changes that occur. Consequently, the sensitivity of a LSPR sensor is enhanced when this distance is minimized by using small size receptor target molecules on the nanoparticle surface (Byun et al., 2013; Park et al., 2014). In a previous study, we demonstrated that binding a small molecule to an aptamer on a GNR surface causes a longitudinal wavelength shift of the LSPR peak as a consequence of a change of local RI induced by formation of a G-quadruplex structure (Park et al., 2014). In contrast to those that are based on antibodies, LSPR sensors that employ small aptamers are expected to have increased sensitivities owing to the fact that binding enables targets to be close to the nanoparticle surface. In addition, a conformational change by the target-binding event of the attached aptamer on the nanoparticle surface should also lead to a local RI change because it can increase the molecular density of sensing volume of the nanoparticle.

In the investigation described below, we designed, fabricated and tested a highly sensitive LSPR-based sensing system, which utilizes a split aptamer to form a sandwich structure, to sense ATP in the form of a large LSPR wavelength shift. Some aptamers can also be folded by two split DNA strands, which is noted as the split aptamer (Cheng et al., 2013; Lin et al., 2010). The use of these types of aptamers for the design of sensors is more flexible because they can have oligonucleotides separated into receptor and probe fragments.

In the newly designed ATP sensor, the receptor fragments are covalently linked to the surface of GNR and the probe fragments have an organic molecule bonded at both ends. Because most organic molecules have higher refractive indices than do buffer solutions, their binding to plasmonic nanoparticles induces an increase in the local RI, which results in a red-shift of the maximum of the LSPR band. TAMRA dye was employed as the organic component in the new ATP sensor system because it can be readily linked to DNA and it has a low molecular weight, small size and high RI compared to those of other biomolecules. It was anticipated that a folded split aptamer complex would form upon binding with ATP and that this event would lead to a shortened distance between the TAMRA dye and the surface of nanostructure. Moreover, higher concentrations of the dye-labeled probe fragments on the nanoparticle surface would result in higher sensitivities owing to the increased density of the sensing volume and change of the RI in the environment of the nanoparticle. These factors would combine to bring about a larger LSPR shift.

The results of the study described below show that the new detection system displays a highly sensitive response to ATP with the detection limit of 10 pM, which is lower than those of previously reported ATP aptasensors (Bao et al., 2015; He et al., 2013; Lu et al., 2015; Song et al., 2014; Wang et al., 2014). In addition, the aptasensor, which can be reused after simple treatment with 2 M NaCl, is applicable to *E. coli* cell analysis. As far as we know, this is the first report describing a LSPR small molecule sensor that takes advantage of a split aptamer assembly and RI induced large LSPR shift in the wavelength of a LSPR signal.

2. Experimental methods

2.1. Reagents

Gold (III) chloride trihydrate ($HAuCl_4$), sodium borohydrate ($NaBH_4$), silver nitrate ($AgNO_3$), L-ascorbic acid, cetyltrimethylammonium bromide (CTAB), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), (3-aminopropyl)trimethoxysilane (APTMS), N,N-dimethylformamide (DMF), succinic anhydride, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), cysteamine hydrochloride, tris (2-carboxyethyl) phosphine hydrochloride (TCEP), magnesium chloride ($MgCl_2$), sodium chloride (NaCl), adenosine 5-triphosphate (ATP), guanosine 5-triphosphate (GTP), cytosine 5-triphosphate (CTP) and uridine 5-triphosphate (UTP) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). An intact, split ATP aptamers and Poly-T were obtained from GENOTECH (Korea).

2.2. Synthesis of gold nanorods (GNRs)

A seed-mediated growth procedure (Nikoobakht and El-Sayed, 2003; Liu and Guyot-Sionnest, 2005) described in our previous report was used to generate GNRs (Park et al., 2014). Briefly, to a seed solution, comprised of 3.75 mL of 0.1 M CTAB and 0.125 mL of 0.01 M $HAuCl_4$, was added 0.3 mL of 0.01 M $NaBH_4$. The growth solution was prepared by slowly mixing 33.04 mL of 0.1 M CTAB, 1.4 mL of 0.01 M $HAuCl_4$, 0.21 mL of 0.01 M $AgNO_3$ and 0.21 mL of 0.1 M ascorbic acid. Then, 0.14 mL of the seed solution was added to the growth solution and the resulting solution was incubated for 3 h. The UV/vis absorption spectrum of the GNR solution was obtained by using a 96 well plate reader (Infinite M200pro, TECAN Group, Ltd., Switzerland). Sizes and aspect ratios were determined by using high resolution transmission electron microscopy (HR-TEM, JEM-2100, JEOL Ltd., Japan; Fig. S1a). The GNRs were found to be uniform with an average aspect ratio of 3.21 (35.85 ± 3.27 nm length and 11.17 ± 1.03 nm width).

2.3. Fabrication of GNR substrate

The GNR substrate was fabricated by using the following, previously described procedure (Byun et al., 2013). Glass slides (10 mm × 10 mm × 2 mm) were cleaned with piranha solution (H_2O_2:H_2SO_4; 3:1 (v/v), this solution is extremely reactive and thus requires handling with extreme care) at 65 °C for 30 min, rinsed with distilled water (DW) and ethanol, and immersed in an ethanolic 2% APTMS solution at room temperature for 1 h. The amine modified slide was treated with 1 M succinic anhydride in DMF at 37 °C for 12 h and then washed with DW. An amide bond forming coupling reaction was carried out by treating the succinic anhydride-modified slide with a 50 mM EDC and 25 mM NHS for 10 min, followed by immersion in 0.1 M cysteamine hydrochloride in DW. Finally, the thiol-modified glass slide was exposed to a solution of GNR for 20 h, which had been centrifuged three times at 10,000 rpm for 15 min each to remove CTAB of GNR. The presence of immobilized GNRs on the glass slide was confirmed using field emission scanning electron microscopy (FE-SEM, S-4700, Hitachi Ltd., Japan; Fig. S1b) and UV–vis adsorption spectroscopy (Fig. S1c). The absorption maximum of the GNR substrate was found to shift to longer wavelength compared to that of a GNR colloid owing to the higher RI of the glass slide compared to that of water and a weak inter-particle coupling effect.