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Direct colorimetric detection of unamplified pathogen DNA by dextrin-capped gold nanoparticles

Amy M. Baetsen-Young[a], Matthew Vasher[b], Leann L. Matta[b], Phil Colgan[a], Evangelyn C. Alocilja[b,*], Brad Day[a,c,**]

[a] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, United States
[b] Department of Agricultural Biosystems Engineering, Michigan State University, East Lansing, MI, United States
[c] Plant Resilience Institute, Michigan State University, East Lansing, MI, United States

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ABSTRACT

The interaction between gold nanoparticles (AuNPs) and nucleic acids has facilitated a variety of diagnostic applications, with further diversification of synthesis match bio-applications while reducing biotoxicity. However, DNA interactions with unique surface capping agents have not been fully defined. Using dextrin-capped AuNPs (d-AuNPs), we have developed a novel unamplified genomic DNA (gDNA) nanosensor, exploiting dispersion and aggregation characteristics of d-AuNPs, in the presence of gDNA, for sequence-specific detection. We demonstrate that d-AuNPs are stable in a five-fold greater salt concentration than citrate-capped AuNPs and the d-AuNPs were stabilized by single stranded DNA probe (ssDNAp). However, in the elevated salt concentrations of the DNA detection assay, the target reactions were surprisingly further stabilized by the formation of a ssDNAp-target gDNA complex. The results presented herein lead us to propose a mechanism whereby genomic ssDNA secondary structure formation during ssDNAp-to-target gDNA binding enables d-AuNP stabilization in elevated ionic environments. Using the assay described herein, we were successful in detecting as little as 2.94 fM of pathogen DNA, and using crude extractions of a pathogen matrix, as few as 18 spores/μL.

1. Introduction

In many parts of the world, emerging diseases account for huge losses in human life, crops, and livestock, and thus, rapid, accurate and reliable monitoring technologies are needed to prevent further impacts on human, plant, and animal health (Foley et al., 2011; Rodrigues et al., 2017). At present, molecular- and biochemical-based techniques, such as PCR and ELISA, are arguably the most reliable methods for the identification of plant and pathogen traits (Kong et al., 2016; Tsui et al., 2011; Wang et al., 2015). Additionally, recent advances in genome-enabled technologies have facilitated the development of probes to rapidly identify genetic markers for trait identification of some of the most devastating pathogens of humans and plants, including *Phytophthora infestans* (potato; Hussain et al., 2014), *E. coli* O157 (Desmarchelier et al., 1998), *Magnaporthe oryzae* (rice; Sun et al., 2015), and *Mycobacterium tuberculosis* (Drosten et al., 2003). However, while PCR-based assays offer sensitivity and specificity, they lack point-of-contact portability and functionality.

In recent years, numerous nanoparticle-based assays have been developed which facilitate the detection of both amplified and purified genomic DNA (Bakthavathsalam et al., 2012; Deng et al., 2013; Zanoli et al., 2012). By exploiting the unique properties of gold nanoparticles (AuNPs), which includes highly specific spectral absorption properties (Sepúlveda et al., 2009), their ability to adhere to DNA, and large surface to volume ratios, AuNPs have emerged as a robust assay for colorimetric biosensing and diagnostics applications, including at single nucleotide polymorphism levels (Daniel and Astruc, 2004; Li and Rothberg, 2004; Upadhyayula, 2012). For example, the use of surface plasmon resonance (SPR) to characterize the interaction between single stranded (ss) and double-stranded (ds) DNA by AuNPs in the presence of salt has illuminated an understanding of the complex association(s) between citrate ions, DNA, and AuNPs (Gearheart et al., 2001; Li and Rothberg, 2004). At a mechanistic level, ssDNA-AuNP interactions are mediated by the stabilization of the nucleotide-nanoparticle complexes in low salt concentrations. In these environments, dsDNA does not adsorb to AuNPs and they therefore aggregate from disruption of SPR. By utilizing these interactions, a variety of DNA nanobioassays have been described, each of which were designed upon salt-induced gold

* Corresponding author.
** Corresponding author at: Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, United States.
E-mail addresses: alocilja@msu.edu (E.C. Alocilja), bday@msu.edu (B. Day).

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nanoparticle aggregation through the use of ssDNA probes (ssDNAp) and un-modified or functionalized AuNPs (e.g., citrate-capped AuNPs (c-AuNPs)) (Fang et al., 2013; Li and Rothberg, 2004a; Torres-Chavolla and Alocilja, 2011; Sattarahmady et al., 2015).

Several technological limitations have prevented widespread adoption of AuNP-DNA nanobiosensors, including the economical and sustainable synthesis of nanoparticles for target detection (Rodrigues et al., 2017). In instances where assays have been developed, the overall detection limits are still relatively low (i.e., ca. 18 ng of genomic DNA) (D'Agata and Spoto, 2012). Additionally, with optimal reaction conditions in the low molar range of salt (i.e., ca. 0.05 M), the use of AuNPs are still limited for most point-of-care assays, as many biological salt concentrations are higher than 0.1 M (Liandris et al., 2009; Munns, 2002, Liu et al., 2015). In recent years, several of these limitations have been resolved, and the use of AuNPs in reaction conditions that parallel native biological conditions have been extended through the use of carbohydrate-coated AuNPs (glyco-AuNPs), which resulted in increased stability and uniformity of the modified nanoparticles, while decreasing the environmental biotoxicity (Anderson et al., 2010; Hastings and Eichelberger, 1937; Wang et al., 2010). In support of greener chemistries, methods have also been developed to synthesize glyco-AuNPs for use in diagnostic applications such as detection of the chemical analyte dihydralazine sulfate by dextran-AuNPs in high ionic biological mediums (Wang et al., 2010). Similarly, a recent study by Torres-Chavolla and Alocilja (2011) demonstrated DNA functionalized dextrin-AuNPs (d-AuNPs) can be used to electrochemically detect the *IS16110* gene from *Mycobacterium tuberculosis* at concentrations as low as 0.01 ng/μL from isothermally amplified DNA (Torres-Chavolla and Alocilja, 2011). Thus, the use of glyco-AuNPs offers the potential for DNA detection in complex biological matrices from demonstrated enhanced stability (Anderson et al., 2010; Torres-Chavolla and Alocilja, 2011; Wang et al., 2010).

In the current study, we used d-AuNPs to detect a unamplified DNA sequence from *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew, currently the primary threat in the United States limiting cucumber production (Summers et al., 2015). As described above, previous reports have demonstrated the use of functionalized d-AuNPs to electrochemically signal DNA target capture; however, the study presented herein represents the first report of colorimetric, sequence-specific, unamplified gDNA detection using unmodified d-AuNPs. To dissect and benchmark this interaction, we compared salt induced aggregation of citrate- and dextrin-capped AuNPs with and without a ssDNAp. These basic explorations into DNA-d-AuNPs interactions enabled us to develop a sequence-specific gDNA-based detection assay utilizing the interactions of ssDNA, dsDNA and d-AuNPs in an elevated ionic environment. Using a combination of UV–vis absorption spectra, aggregation ratios, and transmission electron microscopy (TEM), we have uncovered a putative mechanism underpinning the functionality of this assay. Herein, we propose a model describing the interaction between ssDNA, dsDNA, and d-AuNPs.

2. Material and methods

2.1. Biological reagents

Genomic DNA and sporangia from the plant pathogenic oomycete *Pseudoperonospora cubensis* and its cucumber host, *Cucumis sativus cv* Eureka, were used in this study. The ssDNA oligonucleotide 5′-AATCACAGCTTCTATGTTTTACAT-3′ was synthesized by Integrated DNA Technologies (Coralville, IA). The target sequence of the ssDNAp was 5′- ATG TAA AAC ATA GAA GCT GTG ATT −3′ contained within the genomic DNA of *P. cubensis*.

2.2. Gold nanoparticle synthesis

Dextrin-capped gold nanoparticles (13 nm in diameter) were prepared according to the method of Anderson et al. (2010). In brief, 5 mL of 20 mM $HAuCl_4$ was added to 20 mL of 25 g/L of dextrin stock in a 250-mL flask. The pH of the solution was adjusted to 9.0 with 10% sodium carbonate (Na_2CO_3) and the final reaction volume was adjusted to 50 mL with sterile distilled water (pH 9.0). Particle formation occurred as the flask was incubated at 50 °C for 8 h in the dark. The synthesized nanoparticles were evaluated by TEM using a concentration of d-AuNPs of 7.6×10^{-9} M. This value was derived from Beer's Law based on a molar extinction coefficient of $2.7 \times 10^{8}\ M^{-1}cm^{-1}$ for 13 nm AuNPs (Jin et al., 2003). C-AuNPs (10 nm, 9.93×10^{-9} M) were obtained from Cytodiagnostics (Ontario, Canada).

2.3. DNA extraction

Genomic DNA was extracted from *Pseudoperonospora cubensis* sporangia isolated from cucumber plants using the Machanery Nagel Nucleospin DNA Kit (Düren, Germany). Sporangia were flash frozen in liquid nitrogen and homogenized in a tissue grinder for 40 s at 4.0 M/S using a FastPrep-24 tissue homogenizer (MP-Biomedical, Santa Ana, CA). DNA was purified according to the manufacturer's protocol and quantified by Qubit (ThermoFisher, Waltham, MA). The extracted DNA was stored at −20 °C until use. For non-target DNA reactions, gDNA was extracted from five cucumber leaf discs collected using a #3 cork borer (1 cm^2) and flash frozen in liquid nitrogen. DNA was isolated and purified as described above.

2.4. Assay procedure

To evaluate the stability of the dextrin- and c-AuNPs in the presence and absence of a 66 nM ssDNAp, 20 μL of NaCl (0, 50, 100, 150, 200, 250, and 300 mM final reaction concentration) was added to 10 μL of each of the AuNPs. After a 10-min incubation at 21 °C, the visible absorption spectrum of the d-AuNP aggregation was quantified as described below. ssDNAp-to-target hybridization was initiated by the addition of 2 μL of 1 μM ssDNAp and 5 μL of *P. cubensis* or *C. sativus* extracted gDNA in 3 μL hybridization buffer [10 mM phosphate buffered saline (PBS) at 0.4 M NaCl (pH 7.0)]. Next, the reaction was denatured at 95 °C for 5 min, followed by annealing for 1 min at 57.5 °C. The reaction was cooled for 10 min at 21 ± 1 °C before adding 10 μL of d-AuNPs, followed by 10 μL of 0.8 M NaCl to initiate particle aggregation (NaCl is further denoted as salt). The final reaction solution contained 66 nM ssDNAp, and the concentration of 24 fM *P. cubensis* gDNA or 4 fM of *C. sativus* gDNA, 0.66 nM ssDNAp, 2.5 nM d-AuNPs and 0.3 M NaCl. The reaction was then incubated for 10 min at 21 °C, and the aggregation of AuNPs was quantified by measuring the absorption spectrum of the reaction from 400 to 700 nm.

2.5. Characterization of AuNP aggregation

PCR tubes (200 μL) were used as a reaction vessel. A SpectraMax M2e plate reader (Molecular Devices, Sunnyvale, CA) was used to measure the 520 and 620 nm absorbance values for AuNPs salt and oligonucleotide interactions and genomic DNA sensitivity in a 96 well 200 μL plate. A NanoDrop 2000 (Thermo-Fisher, Waltham, MA) was used to assess the UV–vis absorption spectrum for ssDNA oligomer-to-target hybridization and crude matrix sensitivity. Means of aggregation were separated with a one-way ANOVA using aov in CRAN.R-project. Means were separated at $P \leq 0.05$ using Tukey's honestly significance difference test (R Development Core Team, 2013). Particle dispersion was determined by TEM images and were collected with a JEOL 100CS TEM from 20 μL final reaction volumes containing d-AuNPs in water, in 66 nM ssDNAp, in 66 nM ssDNAp in the presence of 4 fM non-target gDNA, and in 66 nM ssDNAp in the presence of 29 fM target gDNA. All TEM reactions were conducted in 1.5 mM PBS containing 60 mM NaCl. Reactions were incubated at 95 °C for 5 min, followed by annealing for one min at 57.5 °C, and then cooled for 10 min at 21 °C. d-AuNPs