Label-Free Colorimetric Detection of Specific Sequences in PCR amplified DNA

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Abstract:

We observed that single-stranded DNA adsorbs on negatively charged gold nanoparticles (Au-nps) and the adsorption rate is sequence length dependent. We also found that the adsorption stabilizes the Au-nps against salt-induced aggregation. Based on these observations, we developed a simple, rapid and cost efficient colorimetric assay to identify specific sequences in PCR amplified DNA. The assay is label-free without any covalent modification of the DNA or Au-np surfaces involved. The result is displayed with color without need of detection instrumentation. Whole process is completed within 10 minutes.

Keywords: Label-free, colorimetric detection, PCR, DNA sequence, gold nanoparticle

Introduction

Highly selective detection of specific DNA sequences is increasingly important in clinical diagnosis, pathology, and genetics. Recently a wide variety of innovative sensing approaches have been developed, such as surface plasmon resonance (SPR), fluorescent microarrays, assays based on semiconductor or metal nanoparticles, and water-soluble conjugated polymer-based sensors. These techniques have been demonstrated mostly on purified synthesized oligonucloetides, but it may be possible to adapt some of them to be compatible with PCR-amplified samples. Once PCR amplification is utilized, however, the merit of an assay is primarily determined by its simplicity rather than its sensitivity because additional amplification is straightforward. Most of the above approaches require expensive instrumentation or involve time-consuming procudure to modify DNA, substrates, or nanoparticles. In addition, it is usually necessary to conduct hybridization in the presence of substrates that introduce steric hindrance, which leads to slow and inefficient binding between probe and target. As a result, the postprocessing of PCR-amplified samples is expensive and time-consuming.

Recently we observed that single-stranded DNA (ss-DNA) adsorbs to negatively charged Au-nps and the adsorption rate is sequence length dependent. Shorter sequences adsorb faster than longer sequences. We also found that the adsorption can prevent the Au-nps against salt-induced aggregation. Based on these observations, we have designed a rapid colorimetric assay for specific sequences in PCR-amplified DNA based on electrostatics and requires no labeling or surface functionalization chemistry. In our assay, we use the aggregation of unmodified Au-nps as a hybridization indicator. The analytes, PCR product without additional processing, are hybridized with probes under normal physiological conditions prior to exposure to the Au-nps. This avoids the steric constraints associated with hybridization on functionalized surfaces[6] and allows the entire assay including hybridization to be completed within 10 min. The result is determined visually and requires no detection instrumentation.

Results and Discussion

Individual Au-nps have a surface plasma resonance absorption peak at 520 nm and appear pink (Figure 1A, left vial). Immediate aggregation of the Au-nps occurs when enough salt is added to screen the electrostatic repulsion between the Au-nps. The result is blue-gray color (Figure 1A, right vial) characteristic of the surface plasma resonance of Au-np aggregates.

We found that moderate amounts of salt no longer cause aggregation of the Au-nps if enough ss-DNA is added to the gold colloid before addition of the salt that would cause aggregation of the Au-nps without addition of ss-DNA. At the same time, the ability of ss-DNA stabilizing Au-nps against salt-induced aggregation is sequence length dependent. An interesting result is shown in Figure 1B. At current condition, only medium size ss-DNA can stabilize gold colloid against aggregation. The short and long strands do not stabilize Au-nps very well.

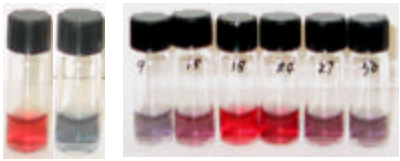


Figure 1, (A) Salt-induced aggregation. Left vial: gold colloid and water, right vial: gold colloid and salt. (B) Sequence length dependent adsorption. The labels on vials are the base number of the sequences

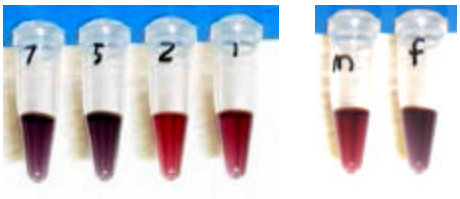
The adsorption of ss-DNA on negatively charged Au-nps seems impossible because ss-DNA is also negatively charged. We can rationalize the fact that ss-DNA sticks to Au-np, as well as the dependence on sequence length, with a simple

picture derived from the theory of colloid science. Both the gold and the ss-DNA attract counterions from the solution and are well described by electrical double layers. In every case, there are attractive van der Waals forces between the oligonucleotide and the nanoparticle. The electrostatic forces are due to dipolar interactions and depend on the configuration and orientation of the ss-DNA. When short segments of the ss-DNA face its bases to the Au-np, attractive electrostatic forces cause ss-DNA to adsorb to the gold. Short ss-DNA sequences are less coiled and are easier to face their bases to the gold. Long ss-DNA sequences usually have more complicated second structure and coiled conformation. Therefore, short sequences adsorb faster than long sequences because long sequences need to break their second structure and uncoil their sequences before the adsorption. The adsorption of ss-DNA to Au-nps increases the charge density on the gold surface, which stabilizes Au-nps against salt-induced aggregation by enhancing the electrostatic repulsion between Au-nps. For the same mole number of ss-DNA, the short strands can not stabilize Au-nps because they do not have enough charges. The long strands can not stabilize Au-nps due to their slow adsorption rate which is resulted from the complicated second structure of long sequences.

The length-dependent adsorption can be used to develop an assay for PCR-amplified DNA sequences that are typically several hundred base pair long. We design short ss-DNA probes which can efficiently stabilize Au-nps against salt-induced aggregation and retain the color of gold colloid. When the probe hybridizes with the long target, they will not adsorb on Au-nps and prevent salt-induced aggregation which changes the color of gold colloid immediately. Based on this idea, we have designed simple colorimetric assays for specific sequences in PCR-amplified DNA. First, we can determine whether the amplified DNA contains the desired sequence by evaluating hybridization with our probes. Second, we can identify single base pair mismatch in the amplified sequences. All of our experiments are performed on PCR product obtained from a clinical diagnosis laboratory without further purification.

We choose two ss-DNA probes with sequences complementary to the desired PCR product and add these to the PCR product solution. The PCR-amplified ds-DNA is dehybridized at 95 °C to produce ss-DNA. These mixtures are annealed below the probe melting temperature so that the probes can hybridize with the PCR-amplified sequence if it is present. When gold colloid is exposed to this mixture, the salt in the hybridization solution causes immediate Au-np aggregation and a color change if the probes have hybridized to the amplified DNA target (Figure 2A, 7 & 5). When the PCR product is not complementary to the probes or the PCR amplification fails altogether, the probes adsorb to the Au-nps and prevent aggregation (Figure 2A, 2 & 1).

We use the same concept as that used for specific sequence detection with a slightly different protocol. Two probes are selected that have the same melting temperature. The sequences are chosen to be complementary to the wild-type sequence of the target. One of the probes bind and overlap the position of the possible point mutation, while one is used as controls and bind at locations that do not overlap the SNP under study. If a mutation exists on the target sequence, the probes covering the mutation will dehybridize at lower temperature than the control probes situated elsewhere in the sequence that are designed to be perfectly matched. At temperatures above where the mismatched sequence dehybridizes but below where the perfectly matched sequence dehybridizes, color differences indicating the presence of a SNP are detected (Figure 2B: m, f).



A B

Figure 2, (A) Label free colorimetric detection for specific sequences in PCR amplified DNA. 7: complementary target; 5: complementary target; 2: non-complementary target; 1: without PCR product. (B) Label free colorimetric detection for single base mismatch in PCR amplified sequences. m: there is a mismatch. f: no mismatch.

Conclusions

We have demonstrated that ss-DNA adsorbs to Au-np with a rate that is sequence length dependent. In addition, adsorption of ss-DNA on Au-np can effectively stabilize the colloid against salt-induced aggregation. We have utilized these observations to design a simple, fast colorimetric assay for PCR-amplified DNA. Our assay obviates the need for gel electrophoresis and other complex sequencing procedures. It can be implemented with inexpensive commercially available materials in less than 10 min, and no instrumentation beyond the programmable thermal cycler used for PCR is required. An important feature of the method is that, unlike chip-based assays or other approaches that utilize functionalized nanoparticles, hybridization occurs under optimized conditions that can be regulated independent of the assay. We believe that our approach can replace some traditional analytical methods for the postprocessing of PCR-amplified DNA and that it will find broad application.

References

1. Li,H.X. and Rothberg,L.J. *PNAS*, **101,** 14036, 2004.
2. Li,H.X. and Rothberg,L.J. *J. Am. Chem. Soc.*, **126**, 10958, 2004.