Rapid DNA sequence identification based on electrostatic interactions with unmodified gold nanoparticles

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Abstract

We find that single and double stranded oligonucleotides have different propensities to adsorb on gold nanoparticles (Au-nps) in colloidal solution. We use this observation to design a hybridization assay for DNA oligonucleotides. Because the underlying adsorption mechanism is electrostatic, no covalent functionalization of the gold or the target DNA is required. Hybridization can be optimized to be rapid and efficient since it is separated from detection. The assay is complete within five minutes and single base pair mismatches are easily detected.

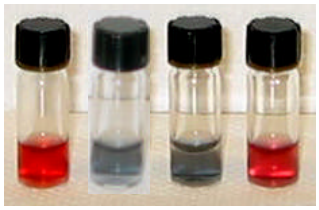
Keywords: DNA sequence, colorimetric detection, electrostatic interaction, gold nanoparticle

Introduction

DNA sequence identification becomes more and more important in personalized medicine, rapid pathogen detection and genetic engineering. Most assays identify specific sequence through hybridization of an immobilized probe to the target that has been modified with a covalently linked label such as a fluorescent or radioactive tag. Oligonucleotide detection protocols that avoid target labeling such as surface plasmon resonance, imaging ellipsometry and chemically functionalized gold nanoparticles have been developed. These methods use complex labelling or surface functionalization chemistry and/or expensive detection instrumentation. Recently we observed that single-stranded and double-stranded oligonucleotides (ss-DNA and ds-DNA) have different affinity to negatively charged gold nanoparticles (Au-nps). Ss-DNA adsorbs to the Au-nps while ds-DNA does not. The adsorption of ss-DNA to the Au-nps further stabilizes the Au-nps against salt-induced aggregation. Based on these observation, we design colorimetric method to sense oligonucleotide hybridization. Our methods are totally derives from electrostatic interaction between DNA and Au-nps so that no modification of the gold or target strands is required. Moreover, hybridization is completely separate from detection so that it can be done under optimal conditions without steric constraints of surface bound probes that slow hybridization and make it less efficient.

Results and discussion

We used a colloidal solution of Au-nps of about 13 nm diameter synthesized via citrate reduction of $HAuCl_4$. Several oligonucleotide sequences and their complements were chosen to emphasize the generality of the method. Attempted hybridization of the probe and the target was conducted in 10 mM phosphate buffer solution containing 0.3 M NaCl at room temperature for 5 minutes. Following the trial hybridization, the trial solution was mixed with gold colloid, and immediately followed by addition of salt/buffer solution. Figure 1 presents color picture of the colloid prior to and after addition of ss-DNA or ds-DNA and salt/buffer solution. Surface plasmon resonance absorption of Au-nps makes the gold colloid display pink color (vial 1). Salt screens the repulsion between the colloidal particles and induces the particle aggregation which significantly changes the surface plasmon resonance absorption and the pink color of colloid into gray (vial 2). Before adding salt to the colloid, we add ds-DNA. It turns out that ds-DNA does nothing to salt-induced aggregation and the mixture displays gray (vial 3). If we add ss-DNA to the colloid before adding salt, the salt can not cause obvious aggregation of Au-nps and the solution retains the pink color of the gold colloid (vial 4).



1 2 3 4

Figure 1, The different abilities for ss-DNA and ds-DNA to stabilize Au-nps against salt-induced aggregation. Vial 1: gold colloid, vial 2: gold colloid + salt, vial 3: gold colloid + ds-DNA + salt, vial 4: gold colloid + ss-DNA + salt.

Based on this observation, we have developed rapid colorimetric assay for specific sequences of DNA oligonucleotides with an idea that once probe hybridizes with target, single stranded probe and target become double stranded. Ds-DNA can not prevent salt-induced aggregation which results in color change of gold colloid. Otherwise, the probe and target stay in single strand which will stabilizes Au-nps from salt-induced aggregation and retains the pink of gold colloid. The procedure of this assay includes:

1, Hybridize probe and target in hybridization solution;
2, Expose the hybridization solution with gold colloid;
3, Add salt to the mixture of 2.

The whole process takes less than 10 minutes without need of any instrumentation. With this procedure we can detection DNA oligonucleotide specific sequences and single base pair mismatches. For the single base pair mismatch detection, dehybridization in water for 2 min is added before exposing hybridization solution to gold colloid in the procedure. Ds-DNA containing mismatch binds weaker and dehybridizes faster in water than perfect matched ds-DNA so that the solution containing mismatched ds-DNA generates more ss-DNA which stabilizes Au-nps against salt-induced aggregation better. Figure 2 shows the results of the detections.

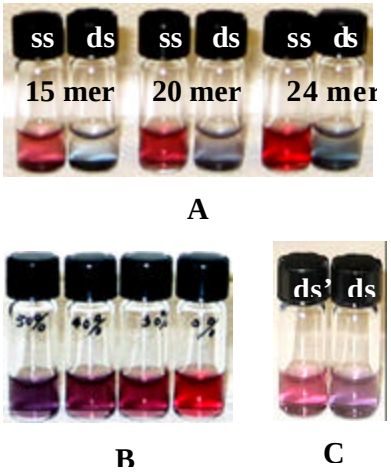


Figure 2, Colorimetric assay for DNA oligonucleotide specific sequences. (A) Three detections for 15, 20 and 24 mer DNA oligonucleotide sequences. (B) Detection for mixtures which contain 50%, 40%, 30% and 0% 24 mer complementary target respectively. (C) Detection for single base pair mismatch in specific sequences. ds' containing single mismatch target, ds containing perfect matched target.

Ss-DNA adsorbs to Au-nps and stabilizes Au-nps against salt-induced aggregation by increasing surface charge density. However, the fundamental issue how negatively charged ss-DNA adsorb to negatively charged Au-np is not clear yet. Ds-DNA is also negatively charged. Why does ds-DNA not adsorb to Au-np? Negatively charged Au-np attracts cations from solution and induces an electric double layer around it. This electric double layer is responsible for the stability of gold colloid. For the same reason, negatively charged DNA backbone also carries an electric double layer. These electric double layers can be simplified as dipoles which point out from Au-np surface or DNA backbone. Short ss-DNA has two possible geometries relative to Au-np, i.e, its backbone facing to Au-np or its bases facing to Au-np. When the bases face to Au-np, the dipole associated with the DNA backbone will be in the same direction with the dipole associated with Au-np surface. These two dipoles in the same direction attract each other. Therefore the dipole attraction and Van der Waals attraction are the reason responsible for the adsorption of ss-DNA to Au-np. While when DNA backbone faces to Au-np, the dipole will point to Au-np and the two dipoles on DNA backbone and Au-np are in opposite direction to each other. These two dipoles repel each other. Especially, once the positive charge layers overlay, big repulsion will dominate Van der Waals force to keep the DNA and Au-np away from each other. Ds-DNA backbone always faces to Au-np so that ds-DNA can not adsorb to Au-np.

Summary

We have demonstrated that ss-DNA and ds-DNA have different propensities to adsorb on gold nanoparticles due to their electrostatic properties. We have used this to design an oligonucleotide recognition assay that uses only commercially available materials, takes less than ten minutes including hybridization, requires no detection apparatus, is sensitive to single base mismatches, and is tolerant of concentration or length mismatches. The assay we have described has additional benefits beyond its speed and simplicity. Because we are able to exploit the electrostatic properties of the DNA, hybridization is separated from detection so that the kinetics and thermodynamics of DNA binding are unperturbed by steric constraints associated with probe functionalized surfaces.

References

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