Biosensors and Bioelectronics 64 (2015) 292–299

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Discovery of the unique self-assembly behavior of terminal suckers-contained dsDNA onto GNP and novel "light-up" colorimetric assay of nucleic acids

Liping Qiu [a,1], Zhifa Shen [c,1], Zai-Sheng Wu [a,b,*], Guo-Li Shen [a], Ruqin Yu [a,**]

[a] State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, People's Republic of China

[b] Cancer Metastasis Alert and Prevention Center, Pharmaceutical Photocatalysis, State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou 350002, People's Republic of China

[c] Shenzhen Kivita Innovative Drug Discovery Institute, Southern District of Science and Technology Park, Nan Shan, Shenzhen 518000, People's Republic of China

ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form
30 August 2014
Accepted 4 September 2014
Available online 6 September 2014

Keywords:
Lying flat-based molecular assembly onto gold nanoparticles
Colorimetric assay
p53 Gene

ABSTRACT

Noble metal nanoparticles are currently of great interest because of their unique optical properties and potential applications in disease diagnostics and cancer treatment. In the present work, a discovery was reported that dsDNA with terminal thiols at its two ends could lie easily flat onto the gold nanoparticle (GNP) surface rather than cross linked different GNPs, indicating an unique self-assembly behavior of newly-designed molecules on GNPs. This could intensively stabilize gold nanoparticles against aggregation even at a high salt concentration. On the basis of this discovery, a novel light-up colorimetric sensing strategy was developed for the detection of p53 gene by combining with the cyclical nucleic acid strand-displacement polymerization (CNDP). For the described colorimetric system, GNPs require no any surface functionalization, and target recognition reaction and CNDP amplification could be conducted under the optimized conditions to achieve a high efficiency. The high detection sensitivity and desirable selectivity are achieved, and the potential practical application was demonstrated. Besides, this sensing system can function in a wide range of salts, making it a suitable platform to cooperate with many biological processes.

1. Introduction

The rapid and reliable detection of specific nucleic acid sequences plays an important role in areas of medical research, clinical diagnosis, and environmental monitoring (Sassolas et al., 2008). Accordingly, various DNA sensors based on the electronic technique (Kwon et al., 2008), mass spectrometry (Hofstadler and Griffey, 2001) and optical measurements (Guo et al., 2009; Shalon et al., 1996; Hou et al., 2014) have been raised. However, most of these suffer from the limitations including high-cost instruments, complicated operation or inadequate sensitivity, making them difficult to prevail particularly in the developing world. The recent advances in the development of nanomaterials have opened up a new chapter for the research of DNA sensors (Sato et al., 2007; Kanjanawarut and Su, 2009; Shen et al., 2012). For example, on the basis of the particular physical and chemical properties of gold nanoparticles (GNPs) (Zhao et al., 2008), numerous colorimetric DNA sensors have been developed and considered as a promising detection strategy for the point-of-care genotyping due to the intrinsic advantages of low cost, rapid response, visible observation and convenient operation (Rosi and Mirkin, 2005).

In 1997, Mirkin and coworkers pioneered a sandwich assay, in which a DNA target molecule cross-links two DNA probe-modified GNPs by hybridization, resulting in the formation of GNP/DNA aggregates accompanied with a red-to-purple color change (Elghanian et al., 1997). Along this line, various GNP-based cross-linking colorimetric assays have been developed for the detection of various analytes, e.g., DNA (Demers et al., 2000; Storhoff et al., 1998, 2000), proteins (Huang et al., 2005) and metal ion (Liu and Lu, 2003, 2004). However, some limitations associated with interface reaction, including complicated operation, time consuming and low efficiency, still exist. Alternatively, based on the fact that single-stranded (ss) oligonucleotides (ssDNA) and double-stranded oligonucleotides (dsDNA) have different ability to protect unmodified GNPs from salt-induced aggregation (H. Li and L. Rothberg, 2004) a non-cross-linking

* Corresponding author at: State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, People's Republic of China. Fax: +86 731 88821916.
** Corresponding author. Fax: +86 731 88821916.
E-mail addresses: wuzaisheng@163.com (Z.-S. Wu), rqyu@hnu.edu.cn (R. Yu).
[1] The two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2014.09.008
0956-5663/

assay for DNA detection was proposed, and then extended to analyze polymerase chain reaction (PCR)-amplified genomic DNA (H. Li and L.J. Rothberg, 2004) and single nucleotide polymorphisms (SNPs) (Cho et al., 2008). Despite of the benefit of speed, simplicity and homogeneous reaction, the assay performance is partly limited by its "light-down" working pattern (hybridization with target triggers GNPs aggregation), the nonspecific DNA adsorption on GNP surfaces and low effect of ssDNA on the stability of GNPs.

In this work, we discovered that two terminal thiols (called TT suckers)-contained dsDNA, which is prepared by hybridization of two complementary 5′-thiol-labeled oligonucleotides, lies flat onto the GNP surface via the thiol–gold self-assembly rather than cross-links two GNPs. Because there are a large number of negative charges in the phosphate backbone of dsDNA, the lying flat of TT sucker-contained dsDNAs leads to the remarkable increase of surface charge density of GNPs. This directly enhances the electrostatic repulsion between the resulting GNPs, protecting the colloidal GNP solution from salt-induced aggregation.

On the basis of this intrinsic self-assembly behavior on GNP surfaces, a light-up colorimetric system for the amplification detection of untagged DNA analytes was proposed via combining with cyclical nucleic acid strand-displacement polymerization (CNDP) (Qiu et al., 2011). A particular codon sequence (273) localized in the exon 8 of the p53 gene (a transcription factor of cell regulation), in which point mutation is the most common phenomenon in carcinogenesis and tumor progression (Marquette et al., 2006), is chosen as the target model. The sensing system developed possesses several advantages such as rapid-response, visible assay, low-cost, and potential in other application ranges. Besides, the hybridization and CNDP amplification completely separated from the detection step can be carried out effectively in homogenous solution without suffering from the limitations of surface functionalization chemistry. Herein, a new working mechanism was demonstrated; the influential factors were explored and the detection performance (e.g., the sensitivity and selectivity) were evaluated. The practicability of this sensing system was also investigated by analyzing the PCR amplicons from p53 gene of Hela cell.

2. Experimental

2.1. Chemicals and materials

Oligonucleotides designed in this study were synthesized by invitrogen Bio Inc. (Shanghai, China), and their sequences are listed in Table 1. All oligonucleotides were dissolved in 4 mM buffer (50 mM Tris–HCl pH 8.0, 4 mM $MgCl_2$) prior to use, unless otherwise stated.

Animal cell genomic DNA extraction kit was obtained from Dingguo Biochemical Reagents Company (Beijing, China). The deoxynucleotide solution mixture (dNTPs) and polymerase Klenow fragment exo$^-$ (5 u/μL) were purchased from Changsha Haiyang Biological Engineering Technology and Services Co. Ltd. (Changsha, China). The polymerase solution was prepared by diluting stock solution with 4 mM buffer to 1 u/μL, unless otherwise stated. Gold nanoparticles (GNPs) (~13 nm in diameter) were prepared following the reported procedure (Yin et al., 2005). The size and dispersity of gold nanoparticles were verified by Transmission electron microscopy (Hitachi TEM 800, Japan). The GNPs concentration (~14.5 nM) was calculated from the UV–visible absorbance of nanoparticle solution at 520 nm with the extinction coefficient of $2.7 \times 108\ M^{-1}\ cm^{-1}$ at λ_{520} for 13 nm particles (Jin et al., 2003). All other chemicals involved were of analytical-reagent grade. Deionized water (resistance > 18.25 MΩ cm) was used throughout.

Table 1
Oligonucleotide sequence (5′–3′) designed in the present study.

Thiolated-recognition probe (HS-RP)	HS-TTTTACCTG CACAAACACGCACCT CGCAGG ACGC
p53 Target DNA (TDNA)	TTG AGG TGC GTG TTT GTG CC
Mutant target DNA (MDNA)	TTG AGG TGC ATG TTT GTG CC
Primer 1	HS-AAA*TCCTGC*
Primer 2	HS-AA*GTCCTGC*
Primer 3	HS-A*CGTCCTGC*
Primer 4	HS-*GCGTCCTGC*
Thiolated-complementary sequence (HS-CS)	HS-GCGT CCTG CGAG GTGC GTGT TTGT G CAGGTAAAA
Recognition probe (RP)	TTTTACCTG CACAAACACGCACCT CGCAGG ACGC
Complementary sequence (CS)	GCGT CCTG CGAG GTGC GTGT TTGT G CAGGTAAAA
Forward primer	CCTGAGGTTGGCTCTGACTG
Reverse primer	AGAGGAGCTGGTGTTGTTGG
Thiolated-stabilizing DNA	SH-TCC TCT CTC TCT CTT TTT T

The two underlined portions of HS-RP are complementary to each other and can form a stem by self-hybridization; and its shaded portion is complementary to the p53 target DNA (TDNA). To prevent TDNA from lengthening during polymerization, one extra nucleotide was designed at the 3'-end of the TDNA. MDNA has the same sequence as TDNA but with a point mutation in the middle. HS-CS is completely complementary to the sequence of HS-RP. Among primer 1, 2, 3, and 4, each has a thiol moiety at their 5'-end and are partially complementary to the 5'-end portion of HS-RP.

2.2. P53 target detection

In a typical experiment, 10 μL of the thiolated recognition probe (HS-RP) (1 μM) (GNP:HS-RP = 1:7) was added to a 200-μL centrifuge tube containing 20 μL of p53 target DNA sample at a certain concentration; then, 10 μL of 1 μM primer 2 (unless otherwise stated, the primer 2 was used throughout) and 5 μL of 1 mM dNTPs were successively injected; after that, 5 μL of Klenow Fragment exo$^-$ (1 u/μL) was added. The reaction solution was incubated at 37 °C for 30 min. Finally, 100 μL of unmodified gold nanoparticle solution was added to the resulting mixture. After incubation for 10 min, and the UV–vis absorption measurement (Shimadzu, Japan) was performed. The ratio of extinction peak at 520 and 700 nm (A_{520}/A_{700}), which was reported to be more accurate for analyte detection (Huang et al., 2005; Liu and Lu, 2003) was used to analyze the experiment data.

2.3. Asymmetric PCR amplification of genomic DNA

Genomic DNA was extracted from Hela cells using the Animal cell genomic DNA extraction kit. PCR amplification was performed in 50 μL of 1× Taq Buffer with $(NH_4)_2SO_4$ containing 2 mM $MgCl_2$, 0.2 mM dNTPs, 3.2 μg of genomic DNA extracted from the cell lines, 1 μM forward primer, 0.2 μM reverse primer, and 1.5 u of Taq DNA polymerase. The amplification process could be summarized as follows: 5 min at 94 °C followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 55 s, then a final extension at 72 °C for 7 min. The PCR products were verified by 2% agarose gel electrophoresis.

3. Results and discussion

3.1. Lying flat-based molecular assembly of TT-suckers-contained dsDNA and improved stability of GNPs

On the basis of the comparative data (seen in Supporting information), the molecular mechanism responsible for the different observations between sample a and b in Fig. 1 is illustrated in Scheme 1A. Although the surface-confined HS-RP is a flexible ssDNA (Scheme 1Aii) and can protect GNPs from aggregation to