Biosensors and Bioelectronics 105 (2018) 137–142

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A simple and non-amplification platform for femtomolar DNA and microRNA detection by combining automatic gold nanoparticle enumeration with target-induced strand-displacement

Tian Li[a,b], Xi Wu[b], Guangyu Tao[b], Haoyan Yin[c], Junlong Zhang[c], Feng Liu[b], Na Li[b,*]

[a] Medical College, Henan University of Science and Technology, Luoyang 471003, China
[b] Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[c] Beijing National Laboratory for Molecular Sciences, State Key Laboratory of Rare Earth Materials Chemistry and Applications, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

ARTICLE INFO

Keywords:
Gold nanoparticle
Dark-field imaging
Enumeration
Nucleic acid
Target-induced strand-displacement

ABSTRACT

By combining the gold nanoparticle (AuNP) enumeration with target-induced strand-displacement reaction, we have developed a non-amplification platform for DNA and miRNA detection based on a deliberately designed sandwich-structured nanocomplex probe (SNC Probe). The proposed strategy can realize the sensitive detection of nucleic acids within 40 min with the detection limit of 6.6 fM for HBV DNA and 13.5 fM for miRNA-141, respectively. The method presents reasonable ability to discriminate miRNA-141 from all the other members of miRNA-200 family. And it can also be used for direct detection of miRNA-141 in samples of extracted total small RNA from different cell lines which are reported to have altered levels of miRNA-141. Furthermore, the spike recovery (n = 3) of miRNA-141 in total small RNA extracts of Hela cells is found to be 92.8% for 20 p.M. and 94.7% for 100 p.M. with the standard deviation of 9.2% and 6.8%, respectively. As the only reagent involved in the assay, the SNC Probe presents a very good stability with a relative standard deviation of 3.3% amongst eight tests in 30 days, which greatly simplifies the assay procedure and presents the suitability for routine analyses. On the basis of these findings, this simple non-amplification assay platform can be expected to find assorted applications that can make the best use of the simplicity and sensitivity.

1. Introduction

The ability for simple and sensitive detection of nucleic acids is a required feature of analytical methods in a variety of applications such as food safety control, environmental monitoring and clinical diagnosis (Du and Dong, 2017; Liang et al., 2014; Smith et al., 2017). To realize the highly sensitive detection, considerable amplification strategies have been developed in the past decades, which often implicate multiple round signal amplification (Liu et al., 2015; Ma et al., 2017; Wang et al., 2017; Zhang et al., 2013; Zhao et al., 2015). However, for practical applications, it is highly desired that an assay involves a minimum number of experimental steps. Particularly, for resource-limited settings, low cost chemical reagents and instrumentation are more of the consideration. In this regard, developing a simple and sensitive non-amplification detection platform is urgently necessary.

AuNPs play an important role in the bioanalytical research and clinical diagnostics due to their outstanding optical attributes benefiting from the localized surface plasmon resonance (LSPR), good biocompatibility and easy surface chemistry (Giljohann et al., 2010; Jain et al., 2008; Zhou et al., 2015). In particular, AuNPs with diameter larger than 40 nm can be easily detected at the single-particle level using the simple and affordable dark-field microscope (DFM) setup with common white-light illumination (Liu et al., 2014b; Yguerabide and Yguerabide, 1998). Analytical methods based on AuNP detection with the DFM is a fascinating optical biosensing technique because of its capability of simultaneously providing multidimensional quantitative information, as well as the high sensitivity, fast detection speed, and the adaptability to a variety of targets of interest (Gu et al., 2015; Huang et al., 2006; Lee et al., 2014; Li et al., 2013, 2017b; Liu et al., 2014a). At present, apart from some works concerning analyzing nanoparticles based on colors of scattered light (Cheng et al., 2014; Jing et al., 2016, 2012; Zhou et al., 2016), most of the AuNP-based dark-field microscopic methods that can achieve the single particle level detection need a spectrometer coupled to the microscope (Chen et al., 2015; Guo et al.,

* Corresponding author.
E-mail address: lina@pku.edu.cn (N. Li).

https://doi.org/10.1016/j.bios.2018.01.034
Received 31 October 2017; Received in revised form 15 January 2018; Accepted 16 January 2018

2013; Lee et al., 2014; Li et al., 2016c; Liu et al., 2012; Poon et al., 2016; Qin et al., 2012; Xiong et al., 2013). In this scenario, the cost of the spectrometer and the complicated procedure to handle the integrated system greatly limit the application in routine analyses.

Single nanoparticle counting, e.g. the AuNP counting, is a simple and cost-effective approach that can achieve high sensitivity without involving the amplification step as well as a spectrometer (Wang and Tao, 2014). Via the specific recognition event, e.g. hybridization or immunological reaction, the target molecules which are invisible with the dark-field microscope can be quantitatively correlated with the number of AuNPs. Previously, we have developed an automatic particle counting method for the single particle level quantification of gold nanomaterials, which can serve as a general sensing platform (Xu et al., 2015). In the subsequent effort, we have successfully demonstrated a non-amplification strategy for femtomolar level DNA detection based on the sandwich-structure formation design (Li et al., 2016b). However, the experimental design does not work well for very short nucleic acids, and more than one separation as well as washing steps is involved, compromising the robustness and simplicity of the assay; furthermore, more examples need to be demonstrated for better understanding of the detection methodology which in turn will facilitate better applications.

In this work, based on the single AuNP enumeration with the dark-field microscope, we present a target-induced strand-displacement based assay strategy for non-amplification detection of DNA and especially the short miRNA which is approximately 19–23 nucleotides. The rationale of the proposed method is illustrated in Scheme 1. The assay relies on a deliberately designed sandwich-structured nanocomplex probe (SNC Probe), which consists of the DNA-functionalized AuNP (AuNP-DNA), linker DNA and DNA-functionalized magnetic bead. The overhang region (black domain) in the sandwich structure serves as a toehold to initiate the strand-displacement reaction for hybridization of the sequence on the AuNP-DNA moiety with the target nucleic acid (Scheme 1A). The lengths of the double helix regions as well as the toehold are designed to assure the occurrence of the target-induced strand-displacement reaction to release AuNPs from the SNC Probe, once the target nucleic acid is introduced. After magnetic separation, the released AuNPs can be enumerated with the dark-field microscope. Since the quantity of the target nucleic acid is highly correlated to the number of released AuNPs, the amount of target nucleic acid can thus be calibrated and determined by counting the number of AuNPs in the dark-field image (Scheme 1B). In the absence of the target, the strand-displacement reaction is much less likely to occur, assuring a low background signal. The transmittance electron microscopic imaging results demonstrate that the strand-displacement reaction can occur, and more released AuNPs are observed with increased concentration of the nucleic acid target (Fig. S1). By using SNC Probe as the only reagent, the proposed non-amplification enumeration strategy can realize the sensitive detection of nucleic acids at the femtomolar level within 40 min with a very simple procedure, thus, should be favorable for practical applications.

2. Material and methods

2.1. Materials

Chloroauric acid ($HAuCl_4$), trisodium citrate, Na_2HPO_4, NaCl, NaH_2PO_4, NaOH, absolute methanol (MeOH), absolute ethanol (EtOH) and NH_2OH• HCl are obtained from Sinopharm Chemical Reagent Co., Ltd. Trimethoxysilylpropyldiethylenetriamine is obtained from J&K Chemical, Ltd. Streptavidin-modified magnetic bead (Dynabeads™ MyOne™ Streptavidin T1, 10 mg/mL) is purchased from Thermo Fisher Scientific. Calf thymus DNA and 6-mercapto-1-hexanol are purchased from Sigma-Aldrich. Tris(2-carboxyethyl)phosphine (TCEP) is purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Diethylpyrocarbonate (DEPC)-treated water are purchased from VWR International Co., Ltd. Coverslips are purchased from Shitai Co., Ltd. (Jiangsu, China). Glass slides are purchased from Matsunami Glass Ind., Ltd. MiRNA isolation kit is purchased from Tiangen Biotech Co., Ltd. (Beijing, China).

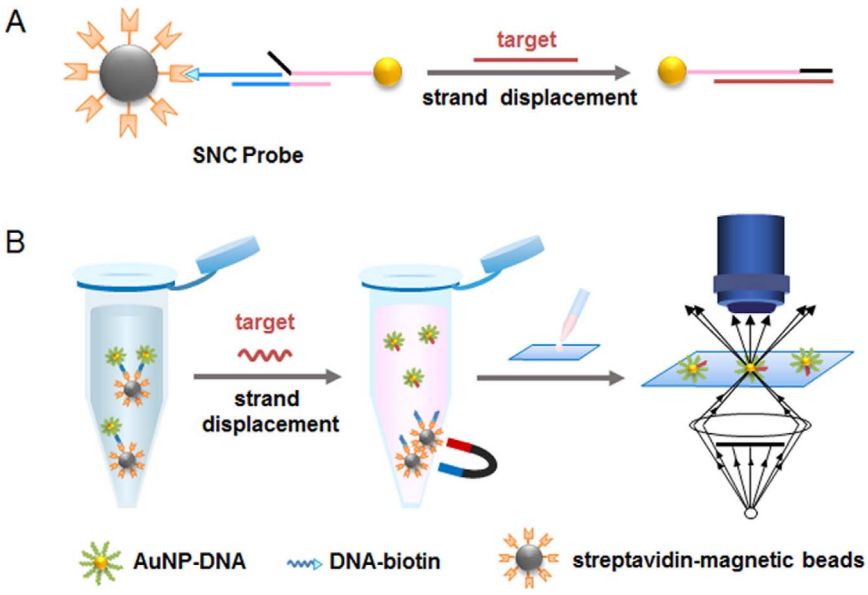
All HPLC-purified DNA oligonucleotides are purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All HPLC-purified miRNA are purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The sequences of the oligonucleotides are listed in Table S1.

2.2. Instrumentation

Ultraviolet-visible (UV–vis) extinction spectra are measured with a Hitachi Model U-3010 spectrophotometer. The size of AuNPs is characterized with a JEM-2100 transmission electron microscopy (JEOL, Japan). The dark-field microscopic images are captured using an Olympus BX-53 microscope with an Olympus DP-72 true color CCD and Olympus cellSense software.

2.3. Modification of glass microscope coverslips

Modification of coverslips is carried out based on reported method



Scheme 1. Schematic illustration of the assay rationale of the proposed method.