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Research paper

A gold nanoparticle-based strategy for label-free and colorimetric screening of DNA triplex binders

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ABSTRACT

A label-free colorimetric assay, using non-crosslinking AuNP aggregation, has been developed for the screening of specific triplex DNA binders. The relative binding affinities can be simultaneously determined. Our novel assay is simple in design and fast in operation, avoiding either AuNPs modification or oligonucleotide labeling, and easy to implement for visual detection. This strategy may offer a new approach for developing low cost, sensitive and high-throughput screening platform that is likely to be highly useful in a wide range of applications.

1. Introduction

The formation of nucleic acid triple helices has been the focus of considerable interest because of possible applications in developing new molecular biology tools as well as diagnostic and therapeutic agents [1–3]. In this strategy, a triplex-forming oligonucleotide (TFO) binds to the major groove of the target duplex in a sequence-specific manner through hydrogen bonding between its bases and exposed groups on the duplex base pairs, generating base triplets. This strategy has proven to be successful in various experimental models, including living cells and animals [4,5], and stable triplexes could provide an effective way to modulate gene expression selectively via transcriptional repression, mutagenesis and recombination [6]. However, triplexes are thermodynamically less stable than the corresponding duplexes, and the poor stability severely restrict the applicability of TFOs in vitro and in vivo [7]. One approach to overcome this limitation is to develop ligands that stabilize otherwise unfavorable triplexes and have a high discrimination between triplex and duplex. In conjunction with TFOs, such ligands will automatically improve triplex stability and also provide additional sequence selectivity, and would open new avenues for the design of antisense, antiviral, and diagnostic agents.

Much progress has recently been made to use small molecules to modulate the properties of triplex structures. A variety of ligands including acridines, naphthoquinolines, BePI, coralyne and neomycin have been identified [8–14]. And recently, we demonstrated the remarkable ability of a small molecule, oxazine 170 to induce formation of a hybrid triplex structure poly rA:(poly dT)$_2$, under solution conditions in which the triplex would not otherwise form [15]. In the past, CD spectroscopy, electrophoresis and UV–vis melting experiments have been used to identify triplex binders [16–18]. However, these protocols are complicated, labor intensive, comparatively slow, and are not well suited for screening large libraries of candidate compounds. Competition dialysis assays that can be used to rapidly screen drug–nucleic interaction have been developed recently, and they still have some limitations [19]. Therefore, a new strategy is needed to overcome these problems for the future development of screening assay.

Herein, we demonstrated a new concept for achieving a potential high-throughput and unmodified gold nanoparticle (AuNP)-based assay for the fast and colorimetric screening of triplex specific binders/inducers. AuNP-based assays have been recently used for the detection of various substances [20–25], based on the high extinction coefficients and unique distance-dependent optical properties of AuNPs. However, most efforts in combining AuNPs with functional DNA, such as the studies of DNA hybridization and ligand binding [26,27], have focused on using functional DNA to

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directly bind to the surface of AuNPs through alkanethiol at the end of functional DNA. In addition, the AuNP aggregation which is induced by inter-particle crosslinking is relative slow process. All of these added the complexity, cost, overall assay time and limited the effectiveness to such detection strategies. Recently, Rothberg and coworkers reported that single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) have different absorption properties on AuNPs [28,29]. ssDNA can effectively bind to AuNPs and stabilize them against salt-induced aggregation. On the other hand, dsDNA which has a rigid structure and exposed negatively charged phosphate backbones can not prevent salt-induced AuNP aggregation. This concept has been used to develop colorimetric method for rapid detection of nucleic acids and extended to enzyme, metal ion sensing and aptamer-based recognition [30–33]. Our strategy was inspired by this non-crosslinking AuNP aggregation phenomenon and the screening process is illustrated in Fig. 1. The assay consists of a duplex DNA and ssDNA (TFO) which has the proper sequence to form a triplex with the dsDNA. In the absence of ligand, the ssDNA adsorbed onto AuNP and prevent the individual red AuNPs from forming blue aggregations under high-ionic strength condition due to the low stability of the triplex structure. However, introducing of a triplex binder/inducer, stabilizes triplex formation through Hoogsteen-type hydrogen bonds. Upon addition of AuNPs, the triplex would not be able to bind and stabilize individual red AuNPs, resulting in purple–blue AuNP aggregations.

2. Experimental section

2.1. Chemicals

Nanopure water (18.2 MΩ; Millpore Co., USA) was used in all experiments and to prepare all buffers. Hydrogen tetrachloroaurate (III) ($HAuCl_4.3H_2O$) was purchased from Acros Organics. Sodium citrate dihydrate was obtained from Alfa Aesar. All DNA binders were purchased from Sigma–Aldrich and used without further purification. All of the oligonucleotides used in this paper were synthesized by Sangon Biotechnology Inc. (Shanghai, China). The sequences are as follows:

DNA-1: 5′-GGAAAAAGAACGGAGGGAGG-3′
DNA-2: 5′-CCTCCCTCCGTTCTTTTTCC-3′
DNA-3: 5′-GGTGGGTGGTTTGTTTTTGG-3′
DNA-4(control): 5′-GTGTGGTGTGTGTTGTGGTTG-3′

2.2. Measurement methods

Electronic absorption spectra and melting analyses were recorded using a CARY 300 UV/Visible spectrophotometer (Varian Inc., Palo Alto, CA), equipped with a thermoelectric temperature controller. A JASCO FP-6500 spectrofluorometer (JASCO International Co., LTD., Tokyo, Japan) was used to measure the light scattering spectra by synchronously scanning the excitation and emission monochromators from 300 to 700 nm (i.e., $\Delta\lambda = 0$). Native PAGE experiments were carried out in 0.045 M Tris–borate buffer. Electrophoresis was carried out by using 15% acrylamide at 80 V for 120 min at room temperature. CD spectra were measured on a JASCO J-810 spectropolarimeter equipped with a temperature-controlled water bath. The optical chamber of CD spectrometer was deoxygenated with dry purified nitrogen (99.99%) for 45 min before use and kept the nitrogen atmosphere during experiments. Three scans were accumulated and automatically averaged. The gels were silver-stained. Photograghs were taken with Canon Ixus 900Ti digital camera.

AuNPs (~13 nm in diameter) were synthesized by means of citrate reduction of $HAuCl_4$. The concentration of AuNPs was

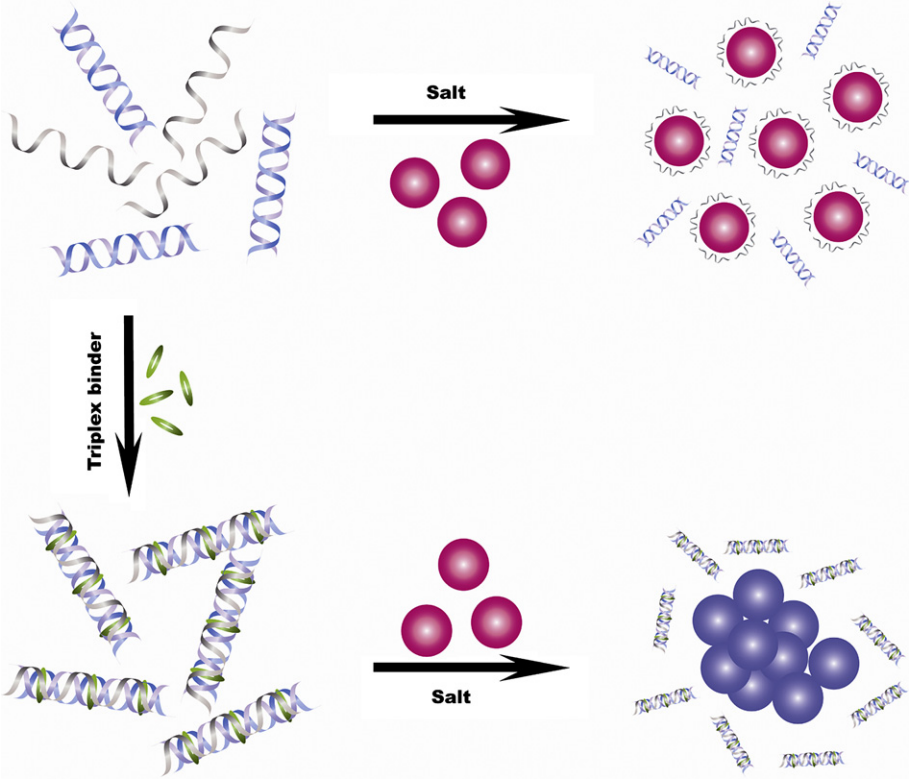

Fig. 1. Schematic illustration of structure and color change of AuNPs-based approach for screening triplex DNA binders.