Sensors & Actuators: B. Chemical 274 (2018) 481–490

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

DNA-silver nanoclusters/polypyrrole nanoparticles: A label-free and enzyme-free platform for multiplexed transcription factors detection

Bingzhi Li[a], Yue Chen[b], Jing Wang[a], Qiaoyun Lu[a], Wanying Zhu[a], Lei Xu[a], Xin Shen[a], Jieping Luo[a], Chunhong Zhu[a], Xiaoxu Li[a], Junli Hong[a], Xuemin Zhou[a,*]

[a] School of Pharmacy, Nanjing Medical University, Nanjing 211166, PR China
[b] Department of Nutrition and Food Safety, School of Public Health, Nanjing Medical University, Nanjing 211166, PR China

ARTICLE INFO

Keywords:
Multiplexed detection
Transcription factors
DNA-silver nanoclusters
Polypyrrole nanoparticles
NF-κB p50
p53

ABSTRACT

Transcription factors (TFs) regulate information flow from gene to protein, and they are recognized as key indicators to reflect cellular processes. Facile monitoring of TFs may aid diagnostics and treatment, but we still lack of techniques for highly efficient detection of them. In this research, a hybrid material consisting of DNA-silver nanoclusters (DNA-AgNCs) and polypyrrole nanoparticles (PPyNPs) was built for TFs detection. The designed DNA-AgNCs have a hairpin-shaped nucleic acid architecture with a double-stranded stem for recognizing TFs and a single-stranded loop for interacting with PPyNPs. In the absence of TFs, DNA-AgNCs are absorbed to PPyNPs, resulting in fluorescent quenching. While in the presence of TFs, the binding between TFs and the DNA-AgNCs caused desorption of DNA-AgNCs via steric hindrance mechanism. Accordingly, the increase of fluorescence derived from desorption is used for quantifications. Derived from low non-specific protein absorption features of PPyNPs, detection limit of 70 pM for NF-κB p50 and 110 pM for p53 were obtained. Then, by absorbing two kinds of DNA-AgNCs to PPyNPs, label-free multiplexed detection of TFs was first realized. Additionally, we suggested that this platform can be developed for drug screening by evaluating inhibitory effect of a pair of optical isomers towards TFs.

1. Introduction

Transcription factors (TFs) are a group of DNA-binding proteins which play key roles in regulating cellular processes [1]. In membrane-nucleus signaling paradigm, when cells are stimulated, signaling pathways are perturbed to make the cells respond to it [2]. The pathways converge on TFs to change their states [3], and then these TFs directly regulate the expression of effector proteins by binding to their binding sites in target genes. The dysregulation of TFs is involved in many diseases, for example the inappropriate regulation of nuclear factor-kappa B (NF-κB) is tightly related to autoimmune diseases, chronic inflammations, pathogen infections, and cancers [4–6]. Another famous TF is p53 which regulates the tumor suppressor gene *p53* [7], and the abnormal alteration of this TF has been found in 50–55% of human cancers [8]. In basic biomedical research, the values of TFs are key indicators to reflect the states of cells or organs [9–11], and it is emerging that TFs could be recognized as diagnostic markers in clinical fields [1,12,13].

Unfortunately, present methods hardly meet our requirements of highly efficient detection of TFs. Lacking of the ability to analyze mass of samples, gel-based methods including electrophoretic mobility shift assay, footprinting, and Western blot are intrinsically limited to research use. Commonly manufactured antibodies usually fail to distinguish activated binding-competent TFs from inactivated binding-inhibited ones [14], which may reduce the specificity of commercial antibody-based methods [15]. To surmount the aforementioned problems, varieties of unconventional methods have been developed in recent years [16]. Based on the attitude to process TFs-DNA binding signal, we can generally divide them into direct and indirect strategies. Indirect ones translate the TFs-DNA binding into release or reserve of DNA fragments, then detect the DNA fragments to indirectly reflect the amount of TFs. Typical examples of indirect strategy include nuclease protection assays [17–20], nucleic acids amplification assays [12,15,21–24]. On the other side, direct ones straightforward recognize

* Corresponding author at: Nanjing Medical University, No. 101 Longmian Avenue, Nanjing 211166, Jiangsu, China.
E-mail address: xueminzhou001_001@hotmail.com (X. Zhou).

https://doi.org/10.1016/j.snb.2018.07.152
Received 24 March 2018; Received in revised form 30 July 2018; Accepted 30 July 2018
Available online 31 July 2018

the TFs-DNA binding and read the signal for detection, representative examples include bimolecular proximity [1,25], conformation switch [13,14], and steric hindrance assays [26,27]. Though sensitive, the indirect assays sometimes prone to low precision because the signal translate progress may reduce their fidelity. Besides, the consumption of extensive regents and enzymes may increase their cost. As to direct strategies, though usually enzyme-free, the reduction of binding constant and loss of TFs-probe binding always leads to limited sensitivity. Therefore, we are still seeking for TFs detection methods which strike a balance between sensitivity and practicability.

DNA-silver nanoclusters (DNA-AgNCs) are collections of fluorescent nanomaterials with diameter of *ca.* 2 nm [28]. Integrating merits of highly fluorescent, photostable, environmental friendly, cost-effective and easy-to-fabricate, DNA-AgNCs have attracted substantial research interest [29]. The most intriguing property of DNA-AgNCs is their aptitude for modular application. The DNA template of DNA-AgNCs contains a nucleation sequence and a functional sequence. By changing the nucleation sequences, the emission of DNA-AgNCs covers the violet to near-infrared [30]. While the functional sequence can be designed as aptamer or complementary sequence for genes or RNA for recognition purpose [31–34]. Therefore, DNA-AgNCs are versatile analytical tools by customizing nucleation and functional sequences according to analytical objectives. However, present applications of DNA-AgNCs mainly focus on aptamer-based recognition and nucleic acids detection. A recent research of our group has first suggested that DNA-AgNCs can be embedded into TFs detection system [35], but actually it was an indirect method which transduced the TFs-DNA binding into a DNA signal and detected the DNA signal to reflect the amount of TFs. Hence, we still lack of DNA-AgNCs-based direct method to detect TFs.

olypyrrole nanoparticles (PPyNPs) are polymerized pyrrole with controllable diameter, good biocompatibility and stability. One of the attractive properties of PPyNPs is that it can absorb near-infrared light and convert the energy into heat, and this makes PPyNPs a promising photothermal therapy agent for cancer treatment [36,37]. Meanwhile, PPyNPs-based biosensing is a growing research area [38]. With extensive five-membered heterocyclic rings on their surface, PPyNPs can specifically absorb ssDNA rather than double-stranded DNA (dsDNA) [39], and it can significantly quench the fluorescence of nearby fluorescein because of energy transfer [40]. Though the selective absorption and quenching properties are similar to other nanocarbons including graphene oxide (GO), PPyNPs show low non-specific protein absorption when compared with others [41]. This outstanding feature can reduce the undesired desorption of DNA probes derived from coexistence biomolecules, minimize the unspecific binding between target and the absorbing surface, and result in increased sensitivity and selectivity. Therefore, PPyNPs-based nanosensors are specifically suitable for detecting biological molecules in complex matrices, especially TFs in cell extracts. Based on these, we attempt to apply PPyNPs in TFs detection for the first time.

In this contribution, we integrate DNA-AgNCs with PPyNPs to build a hybrid platform (DNA-AgNCs/PPyNPs) for TFs detection. The DNA-AgNCs are formed in hairpin shape, and their loop domain are absorbed to the PPyNPs, resulting in fluorescent quenching. In the presence of target TFs, the binding event between TFs and the stem domain of DNA-AgNCs forces the DNA-AgNCs desorbed from the surface. The desorption is derived from steric hindrance, and it leads to fluorescent recovery for detection. In contrast to former reported steric hindrance assays, our method allows for label-free and facile detection of TFs with increased sensitivity. We further utilized the tunable fluorescence of DNA-AgNCs to realize multiplexed detection of two TFs in one system for the first time. Overall, combining the advantages of DNA-AgNCs and PPyNPs, we built a highly efficient platform for TFs quantification.

2. Materials and methods

2.1. Materials

HPLC-purified oligonucleotides were ordered from Sangon Biotech (Shanghai, China), and their sequences were presented in Table S1. $AgNO_3$ (metal basis, 99.999%) and analytical grade reagents including $NaBH_4$, Polyvinylpyrrolidone (PVP, MW = 24,000), Pyrrole and $FeCl_3{\cdot}6H_2O$ were purchased from Aladdin Reagent (Shanghai, China). Buffers were prepared using molecular biology grade salts obtained from BBI (Shanghai, China). Native polyacrylamide gel (native-PAGE) was prepared by electrophoresis grade reagents purchased from Macklin (Shanghai, China). Purified human recombinant NF-κB p50 (untagged, ~41 kDa), NF-κB p65 (untagged, ~35 kDa), and p53 (GST-tagged, ~82 kDa) were obtained from Enzo Life Sciences (NY, U.S.A). The left and right-handed Dehydroxymethylepoxyquinomicin ((−)-DHMEQ and (+)-DHMEQ) were purchased from MedChem Express (NJ, U.S.A.). Ultrapure water were produced by a Millipore's Synergy system and treated with DEPC (Sangon Biotech, Shanghai, China) before use.

2.2. Preparation of DNA-AgNCs

DNA-AgNCs were prepared according to previous literatures [42–44]. Typically, 1 OD of DNA template was dissolved to 15 μM by citrate buffer (10 mM, pH = 6.8).The solution was heated to 95 °C for 10 min, and then slowly cooled down to room temperature for 1 h. Then, 1 mM of $AgNO_3$ was added to the solution, and the reaction was kept at room temperature for 15 min. Freshly prepared 1 mM of $NaBH_4$ was quickly added to the mixture followed by 5 min of vigorous shaking, and then incubated at room temperature overnight before use. The whole reaction should be conducted in a dark place, and the final concentration ratio between DNA template, Ag^+ and BH_4^- was 1:6:6 for red-emitting DNA-AgNCs probe-1(AgP1, λ_{em} = 627 nm), and 1:8:4 for near-infrared-emitting DNA-AgNCs probe-2 (AgP2, λ_{em} = 833 nm).

2.3. Preparation of PPyNPs

PPyNPs were synthesized and purified following previous reports with modifications [41,45]. Briefly, 2 g PVP was dissolved in 50 mL of water. After stirring for 30 min, 250 μL of pyrrole monomer was added to the solution and dispersed for 15 min. Then, polymerization was initiated by adding $FeCl_3{\cdot}6H_2O$ (2 mL, 0.75 g/mL) to the reaction drop by drop. After 3 h of reaction, the product was washed by copious amount of methanol-acetone (v/v = 1:1) mixture and centrifuged at 16,000 rpm for 10 min for 6 times. The obtained precipitate was dispersed in water and dialyzed in dark place using a 25 kDa MWCO dialysis bag against ultrapure water for 48 h with water change every 6 h.

2.4. Cell culture and treatments

Human colon cancer DLD-1 cells were obtained from the American Type Culture Collection and maintained in RPMI-1640 with 10% fetal bovine (Gibco, U.S.A.) and cultivated with 5% CO_2 at 37 °C. To stimulate the cells, H_2O_2 (BBI, Shanghai, China) or TNF-α (PeproTech, Rocky Hill, NJ) was added to the cells. The NF-κB p50 knockdown assay was conducted using siRNA (SC-29407, Santa Cruze, U.S.A.) according to instructions provided by the manufacturer. Nuclear extracts were harvested by a nucleoprotein extraction kit (Sangon Biotech, Shanghai, China). The total protein concentration of all samples were unified using a Bradford-based test kit (GeneRay Biotech, Shanghai, China) before further analysis.