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Counterions-mediated gold nanorods-based sensor for label-free detection of poly(ADP-ribose) polymerase-1 activity and its inhibitor

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ABSTRACT

Stable and sensitive detection of poly(ADP-ribose) polymerase-1 (PARP-1) is of great significance in many fields. Herein, a highly simple and sensitive colorimetric biosensor for label-free detection of PARP-1 based on counterions-induced gold nanorods (AuNRs) aggregation is put forward in this work. The specific DNA activated PARP-1 and resulted in the formation of electronegative poly(ADP-ribose) polymer (PAR) on the auto-modified domain of PARP-1 when nicotinamide adenine dinucleotide (NAD^+) was used as substrate. Negative charged PAR induced the aggregation of CTAB-coated AuNRs which are employed as the colorimetric probe here, resulted in the obvious decrease of absorbance and the vivid color change. The aggregation process was visually observed by transmission electron microscope (TEM), and dark-field measurements (DFM). By utilizing the change of longitudinal absorption and vivid color variation of AuNRs, PARP-1 can be detected in a linear range of 0.05–1.0 U with a detection limit of 0.006 U (0.261 ng), which is two orders of magnitude improved compared with previously reported colorimetric methods based on AuNPs. This detection method is label-free, visualized and reliable. The used AuNRs probe is stable and their synthesis procedures are simple. The method has been used to detect PARP-1 in ovarian cancer cells A2780, human breast cancer cells SK-BR-3 and MCF-7, obtained with satisfactory results. Besides, it has also been applied to evaluate the PARP-1 inhibitors, detect the change of PARP-1 activity in the presence of various DNA damages.

1. Introduction

Poly(ADP-ribose) polymerases (PARPs) are a superfamily of enzymes related to post-translational modification of protein present in most eukaryotic cells. PARP-1, one of the main member of the PARP family, is the best characterized enzyme. Studies showed that PARP-1 was implicated in multiple processes which involved DNA-related transactions. So, it was also named as the "guardian angel of DNA" by P.A. Jeggo [1–3]. The level of PARP-1 in cells was not only closely related to certain types of cancer but also dramatically affected the selectivity and efficiency of PARP-1 inhibitors in proliferous cancer cells [4]. Therefore, the study of PARP-1 activity and PARP-1 inhibitors plays an important role in cancer treatment.

Stimulated by DNA damage, PARP-1 is rapidly activated and then nicotinamide adenine dinucleotide (NAD^+) is catabolized into ADP-ribose unit and nicotinamide [5,6]. Under the catalysis of activated PARP-1, the ADP-ribose chain can grow up to 200 units by repeated attachment of ADP-ribose unit, which is named PARylation. In addition to linear extension, the PAR polymer also forms branches composed of 20–50 ADP-ribose units [7,8]. Many studies on PARP-1 activity detection and its inhibitor evaluation were based on the unique property of PARylation. Among these methods, introducing isotope-labeled NAD^+ into PARP-1 homodimers is the main methods. Unfortunately, this type of method possesses the disadvantages of being time-consuming, requiring of substrate radiolabeling or involving complicated procedures and sophisticated apparatus. In order to avoid these problems, other methods have also been developed. For instance, Xu put forward a colorimetric method based on the aggregation of gold nanoparticles [9]. However, the method is not very sensitive because it is based on the detection of consumed NAD^+ and the detection limit is 0.8 U

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(36 ng), which may limit the application of the method in real sample detection.

Colorimetric methods possess plenty of advantages such as instrument-free, cost-effective, and always accompany with a vivid color change that can be easily observed by the bare eyes. So, an inexpensive, selective and enzyme-free colorimetric method based on AuNRs has been proposed for the quantitative determination of PARP-1. Gold nanorods (AuNRs), with size-controlled optical properties and favorable biocompatibility [10], have been widely employed for the applications of cell imaging [11], cancer diagnosis [12], medical photothermal therapy [13], and detection of various proteins [14]. Specially, AuNRs have been used on multifarious colorimetric biosensors because they possess strong localized surface plasmon resonance (LSPR) that enables them to absorb the light effectively range from visible to near-infrared (near-IR) region [15]. Moreover, upon aggregation, the absorption efficiency of the longitudinal absorption peak decreased obviously due to the dielectric properties of the surroundings and external environment of the AuNRs, and usually accompanying with a slightly red-shift which probably resulted from the plasmon coupling between contiguous rods [16,17].

Inspired by the unique optical properties of AuNRs and the large negative charge density of PAR that formed under the catalysis function of PARP-1, a label-free colorimetric method for the visualized and precise detection of PARP-1 activity and its inhibitors has been proposed. PAR that produced by PARP-1 contains an adenine moiety with two phosphate groups that carry negative charges. Because of diverse elongation and branching process, PAR forms strongly negatively charged heterogeneous polymers both in vitro and in vivo. These negatively charged heterogeneous polymers accelerated the aggregation of CTAB-coated AuNRs that have ample positive charges, which led to the decrease of longitudinal absorption and the obvious color variation of AuNRs. PARP-1 was sensitively detected by ultraviolet spectrometer. Meanwhile, it can also be discerned by bare eyes. The used probe of AuNRs are stable and their synthesis procedures are simple. So, this detection method is label-free, visualized, simple and reliable. Limit of detection is two orders of magnitude improved compared with previously reported colorimetric methods based on AuNPs. The method has been used to detect PARP-1 in ovarian cancer cells of A2780, human breast cancer cells of SK-BR-3 and MCF-7 and human serum samples, obtained with satisfactory results. Besides, it has also been used to evaluate the PARP-1 inhibitors and detect the change of PARP-1 activity in the presence of various DNA damages. All these findings proved that the proposed colorimetric method offered a convenient, high-efficient and inexpensive platform for the detection of PARP-1 activity and evaluation of its inhibitors.

2. Experiment section

2.1. Materials and reagents

Chloroauric acid ($HAuCl_4$), cetyltrimethylammonium bromide (CTAB), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), hydroquinone were purchased from Sinopharm Chemicals Reagent Co. Ltd (Shanghai, China). Bovine serum albumin (BSA) from human plasma were obtained from healthy donors at the Second Affiliated Hospital of Southeast University (Nanjing, China). Human PARP-1 was purchased from Trevigen (Wuhan, China). Benzamide (an effective PARP-1 inhibitor), NAD^+ were purchased from Sigma-Aldrich (Shanghai, China). Rucaparib phosphate (AG014699) was obtained from the company of MedChemExpress (New Jersey, USA). All solutions were prepared using ultrapure water (18.2 MΩ cm at 25 °C) from a Milli-Q water purification system (Barnstead, Thermo Scientific, USA). S-adenosylmethionin (SAM), *E. coli* CpG methyltransferase (M. SssI) were obtained from New England BioLabs (Ipswich, MA). Nuclear and Cytoplasmic Protein Extraction Kit was obtained from Beyotime Biotechnology (Shanghai, China).

The G-quadruplex DNA, activated DNA, and the specific DNA for methylation (T1) were all synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). Their sequences were showed as Table S1. Buffer solutions employed in this study were as follows. DNA hybridization buffer (H-buffer) contained 10 mM Tris–HCl, 0.1 M NaCl (pH 7.4). The compositions of G-quadruplex formation buffer (F-buffer) were 50 mM Tris–HCl, 100 mM KCl (pH 7.4). The reaction buffer (R-buffer) was composed of 50 mM Tris–HCl, 2 mM $MgCl_2$, 50 μM Zn $(OAc)_2$, and 50 mM KCl (pH 7.4). DNA methylation buffer (M-buffer) contained 50 mM Tris–HCl, 50 μM SAM, 5 mM $MgCl_2$, 0.1 mg mL^{-1} BSA and 1 mM DTT (pH 7.9).

2.2. Instrumentation

A UV–vis spectrophotometer (Cary 100, Agilent, Singapore) was used to record UV–vis absorption spectrum. Transmission electron microscopy (TEM) images were collected on a transmission electron microscope (JEM-2010, Hitachi, Japan) with an acceleration voltage of 200 KV. Before running TEM tests, 10–15 μL of each dispersive sample solution was first dropped onto a copper grid, and then dried at room temperature. The dark-field measurements (DFM) were achieved on a Nikon inverted microscope Eclipse Ti-U equipped with a dark-field condenser ($0.8 < NA < 0.95$), a 60× objective lens ($NA = 0.7$) and a color charge coupled device (CCD, S45, Canon, Japan) to record the color dark-field images. The zeta potentials of each sample were measured by Zetasizer Nano S/ZS (Malvern, UK) at 25 °C and measurements were performed at least three times after dilution in deionized water. The zeta potentials were calculated using the Brookhaven Dispersion Technology Software.

2.3. Synthesis of AuNRs with different longitudinal plasmon bands

AuNRs were prepared by chemical reduction process following the one-pot synthetic method according to the previous literature [18,19]. AuNRs were synthesized by adding sodium borohydride into a growth solution containing the gold ion precursor of $HAuCl_4$, silver nitrate, CTAB, hydroquinone, and sodium borohydride. Firstly, $HAuCl_4$ was rapidly added into CTAB solution with gentle shaking. The color of the solution quickly changed from transparent color to dark yellow. Subsequently, $AgNO_3$ and hydroquinone were successively added into the solution for the incubation of 5 min. The color of the solution changed from dark yellow to pale yellow and finally to colorless. Finally, freshly prepared ice-cold $NaBH_4$ solution was dropped into the mixed solution, accompanied by gently shaking for about 10 s, to trigger the growth of AuNRs. The growth solution was maintained at 30 °C in a water bath for over 12 h without disturbance.

The obtained AuNRs solution was centrifuged at 13,000 rpm (15.7*g*) and 20 °C for 20 min. Subsequently, the supernatant liquid was carefully discarded to remove supernumerary CTAB and small spherical nanoparticles. After twice purifications, the obtained precipitations were redispersed with ultrapure water. Then, different AuNRs with the longitudinal plasmon wavelength of around 635 nm, 675 nm, 745 nm, and 800 nm were obtained, which was strongly dependent on the $AgNO_3$ concentration.

2.4. Preparation of G-quadruplex DNA and activated DNA and T1

According to the previous reported procedures [20], 3 μL of G-quadruplex DNA (50 μM) was mixed with 147 μL of F-buffer, followed by heating to 95 °C for 5 min and then naturally cooling down