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Self-templated formation of aptamer-functionalized copper oxide nanorods with intrinsic peroxidase catalytic activity for protein and tumor cell detection

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ABSTRACT

We have synthesized aptamer-functionalized copper nanoparticles (Apt–Cu NPs) using simple ascorbate-mediated reduction of Cu(II) ions in Apt-T_n templates comprising an aptamer (Apt) sequence with a poly(dT) motif at both the 3′- and 5′-termini. The as-prepared Apt–Cu NPs underwent rapid oxidation to Apt-T_n–CuO/Cu_2O NPs. The CuO/Cu_2O NPs tended to have rod-like shapes when the Apt-T_n templates had long poly(dT) units ($n > 30$). Interestingly, the as-formed Apt-T_n–CuO/Cu_2O NPs exhibited intrinsic peroxidase-like activity for the H_2O_2-mediated oxidation of Amplex Red (AR) to fluorescent resorufin. The catalytic activity of thrombin binding aptamer-templated CuO/Cu_2O NPs (TBA-T_n–CuO/Cu_2O NPs) was significantly suppressed after the specific binding of thrombin to the aptamer units on the particles' surfaces. The H_2O_2/AR–TBA-T_n–CuO/Cu_2O NP probe provided a limit of detection (LOD) of thrombin in serum samples of 0.5 nM. Using Mucin1 binding aptamer-templated CuO/Cu_2O NPs (Apt_{MUC1}-T_{30}–CuO/Cu_2O NPs), we applied this sensing platform to the detection of Mucin1-overexpressing tumor cells (LOD: ca. 100 cells). This study suggests that the Apt–T_n can be used as a universal template for preparation of enzyme-like CuO/Cu_2O NPs for detection of proteins and labeling of tumor cells when using appropriate aptamers.

1. Introduction

Enzyme-like nanomaterials (nanozymes) [1,2] based on metallic [3–5], metal oxide [6–10], bimetallic nanoparticles (NPs) and nanoclusters (NCs) [11–14], carbon nanomaterials [15–17], and hybrid nanocomposites [18–25], have recently received a great deal of attention for their biochemical applications. Enzyme-mimicking molecules (e.g., metal complexes, hemin and other porphyrins, cyclodextrins, and supramolecules) also have been reported to have many advantages over natural enzymes [26–28]. Enzyme-like nanomaterials can display superior properties and have several advantages such as high stability against harsh conditions, ease of preparation, facile structural control (tunability of catalytic activity), purification, recovery, recycling and storage; large-scale synthesis, and low cost. Moreover, the crystallinity and surface properties of nanomaterials, including variations in their species, charges, vacancies, and oxidation states, can be exploited to alter enzymatic oxidase, peroxidase, catalase, superoxidase dismutase, nitrate reductase, phosphatase, and glucose oxidase activities.

Many nanomaterials have also displayed stable and high enzyme-like activity, thereby allowing their application in bioassays for glucose, DNA, and proteins through quantitation of H_2O_2 [1,2,29]. In addition, catalytic NPs have been modified with small molecules and antibodies for immunohistochemical assays of specific proteins and tumor cells [9,19,20]. Nevertheless, the catalytic activity of label-free (unmodified) NPs typically decreases dramatically, due to nonspecific interactions with biomolecules (e.g., proteins) in biological samples. Moreover, the catalytic activities of

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modified enzymatic NPs also typically decline due to a decrease in surface vacancies. Therefore, one-step synthetic processes for the preparation of enzyme-like nanomaterials with self-assembling functional ligands on the particle surfaces are in high demand for bio-applications.

In this study, we developed a facile, one-step strategy for rapid synthesis of DNA-templated copper nanoparticles (Cu NPs) that display selective targeting and highly efficient peroxidase-like catalytic activity. TBA_{29}-T_n, with poly(dT) (T_n) templates at both the 3′ and 5′-termini of a 29-mer thrombin-binding aptamer (TBA_{29}) were used for the preparation of these Cu NPs, where TBA_{29} [30] has the additional advantage of selective binding to thrombin (Scheme 1). Synthesis of the Cu NPs using poly(dT) as a template involves high-affinity binding of thymine and Cu^{2+}, and subsequent chemical reduction (via ascorbic acid) of the Cu^{2+}—thymine complexes to Cu^0, resulting in the formation of Cu NPs along the poly(dT) scaffolds [31]. TBA_{29}-T_n–Cu NPs fluoresce according to the quantum size effect; due to oxidation mediated by dissolved molecular oxygen (O_2), the fluorescence of the TBA_{29}-T_n–Cu NPs decayed rapidly as TBA_{29}-T_n–CuO/Cu_2O NPs were formed in solution. Nonetheless, the TBA_{29}-T_n–CuO/Cu_2O NPs exhibited strong peroxidase-like activity originating from the Cu^+/Cu^{2+} ions on the particle surfaces, and could be employed to detect thrombin (Scheme 1), which inhibits the catalytic activity of H_2O_2-mediated oxidation of Amplex Red (AR, 10-acetyl-3,7-dihydroxyphenoxazine) to fluorescent resorufin (7-hydroxy-3*H*-phenoxazin-3-one) [32]. Thrombin (activated blood-coagulation factor II) is a key terminal enzyme in the coagulation cascade that converts soluble fibrinogen into insoluble strands of fibrin, and also catalyzes many other coagulation-related reactions (e.g., direct activation of protein C and platelets) and feedback activation of procofactors V and VIII [33]. In this paper, we demonstrate the use of AR/H_2O_2–TBA_{29}-T_n–CuO/Cu_2O NP probes for the analysis of thrombin-spiked serum samples. We have also employed our sensing platform, using Mucin1 binding aptamer-templated CuO/Cu_2O NPs, for the detection of breast tumor cells.

2. Preparations and methods

2.1. Preparation and characterization of TBA_{29}-T_n–Cu NPs

A solution of TBA_{29}-T_n (625 nM) in 12.5 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer (1.6 mL, pH 7.4) and 125 mM NaCl was mixed with freshly prepared ascorbic acid solution (50 mM, 0.2 mL) at room temperature. Copper(II) nitrate solution (5 mM, 0.2 mL) was introduced to trigger the formation of fluorescent Cu NPs. After incubating for 10 min, the solution was transferred separately to a 96-well microtiter plate, and then the absorption and fluorescence spectra (excitation wavelength: 340 nm) were recorded using a monochromatic microplate spectrophotometer (Synergy 4 Multi-Mode Reader, BioTek, Winooski, VT, USA).

2.2. TBA_{29}-T_{30}–CuO/Cu_2O NP probes for thrombin detection

Aliquots (400 μL) of a physiological solution containing 31.2 mM Tris-borate buffer (pH 7.4), 187.5 mM NaCl, 6.25 mM KCl, 1.25 mM $MgCl_2$, 1.25 mM $CaCl_2$, 125 μM bovine serum albumin (BSA), TBA_{29}-T_{30}–CuO/Cu_2O NPs (62.5 nM) and thrombin (0–125 nM) were equilibrated at room temperature for 1 h. H_2O_2 (50 mM, 50 μL) and AR (100 μM, 50 μL) were then added, and fluorescence was measured after 2 h (microplate spectrophotometer; excitation wavelength: 540 nm).

2.3. Cell cultures and detection of tumor cells

A breast tumor cell line (MCF-7), a transformed human embryonic kidney cell line (293T), and a hepatocellular carcinoma cell line (HepG2) were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in alpha-MEM medium containing 10% fetal bovine serum. All cells were cultured in a humidified incubator at 37 °C under 5% CO_2. The preparation procedure for the MUC1 aptamer–functionalized CuO/Cu_2O NPs (Apt_{MUC1}-T_{30}–CuO/Cu_2O NPs) was the same as that described for the TBA_{29}-T_{30}–Cu/Cu_2O NPs. For the detection of tumor cells, the cells were cultured on 8-mm chips in 48-well plates for 8 h at 37 °C. The cultured cells were labeled separately with Apt_{MUC1}-T_{30}–CuO/Cu_2O NPs (200 nM) for 1 h in physiological buffer. The cell-adhesive chips were then removed and washed three times with PBS, and reacted with AR (10 μM) and H_2O_2 (5.0 mM) for 1 h. Finally, the fluorescence of resorufin (oxidized AR) in each solution was measured using a microplate spectrophotometer (excitation wavelength: 540 nm).

See the Supplementary Information for the details on the materials, characterization of TBA_{29}-T_n–Cu NPs, electrochemical impedance spectroscopy (EIS), and detection of thrombin in serum samples.

3. Results and discussion

3.1. TBA_{29}-T_n–Cu NPs oxidized to TBA_{29}-T_n–CuO/Cu_2O NPs

We synthesized TBA_{29}-T_n–Cu NPs through simple ascorbate-mediated reduction of Cu^{2+} ions in the presence of TBA_{29}-T_n templates. Binding of Cu^{2+} ions with TBA_{29}-T_n templates with different poly(dT) motifs at both the 3′- and 5′-ends (sequences listed in Table S1, Supplementary data) produced TBA_{29}-T_n–Cu NPs of various sizes and shapes. XPS (Fig. S1, Supplementary data) and XRD (Fig. S2, Supplementary data) studies indicated that the prepared TBA_{29}-T_n–Cu NPs were readily oxidized to TBA_{29}-T_n–CuO/Cu_2O NPs. In addition, TEM images (Fig. 1a) revealed that the TBA_{29}-T_6–CuO/Cu_2O NPs and TBA_{29}-T_{15}–CuO/Cu_2O NPs were spherical, whereas the TBA_{29}-T_{30}–CuO/Cu_2O NPs had short rod-like shapes (aspect ratio: ca. 2.2). From TEM analyses, we calculated mean particle sizes of the TBA_{29}-T_6–CuO/Cu_2O, TBA_{29}-T_{15}–CuO/Cu_2O, and TBA_{29}-T_{30}–CuO/Cu_2O NPs of 2.17 ± 0.29, 3.21 ± 0.56, and 4.20 ± 1.58 (diameter)/9.15 ± 2.86 (length) nm, respectively. In contrast to the spherical shapes of single-stranded poly(dT)- or double-stranded DNA-templated Cu NPs, our TBA_{29}-T_{30}–CuO/Cu_2O NPs had a rod-like shape [31,34–37]. The rigidity of the G-quadruplex structure of TBA_{29} led to the low flexibility of the terminal poly(dT) units. Growth of these Cu NPs with low flexibility and long poly(dT_{30}) resulted in the formation of rod-like structures that have a mean length of approximately 9.2 nm; similar to that of poly(dT_{30}) (ca. 9 nm), which indicates growth of the Cu NPs along the T_{30} unit.

UV–vis absorption analysis of these freshly prepared Cu NPs indicated similar absorptions at 345 nm (Fig. S3a, Supplementary data), consistent with the spectra of previously reported fluorescent Cu NPs synthesized using ds-DNA or poly(dT) templates [31,34–37]. However, the fluorescence of the TBA_{29}-T_{30}–Cu NPs at 650 nm (excitation at 340 nm) was much stronger than that of the TBA_{29}-T_6–Cu NPs and TBA_{29}-T_{15}–Cu NPs (Fig. S3b, Supplementary data). The fluorescence quantum yield (QY) of the freshly prepared TBA_{29}-T_{30}–Cu NPs was approximately 1%, measured in comparison with quinine (QY: 53%). In contrast, the Cu NPs prepared without templates or with TBA_{29} having poly(dA_n) or poly(dC_n) ($n = 6$, 15, 30) at both the 3′- and 5′-termini had sizes larger than 10 nm and exhibited very weak fluorescence (QY < 0.01%) and very