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ORIGINAL PAPER

Synthesis of DNA-templated copper nanoparticles with enhanced fluorescence stability for cellular imaging

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Abstract

Fluorescence of DNA-templated copper nanoparticles (DNA-CuNPs) is not stable over time which limits applications in cellular imaging. This is due to the presence of oxygen during synthesis which oxidizes Cu(0) to Cu(II) and also produces the free hydroxyl radical. The authors have prepared DNA-CuNPs with enhanced temporal stability of fluorescence by optimizing the reaction conditions so as to minimize the deleterious effects of oxygen. The operational lifetime of DNA-CuNPs was increased from 25 min to 200 min. Fluorescence spectra of DNA-CuNPs in optimized condition show an emission peak at 650 nm when excited at 340 nm. DNA-CuNPs synthesized in this manner were used for cell imaging. As a proof of concept, the nucleus of a human colon cell line (HCT116) was stained. The method does not involve any chemicals other that copper sulfate and ascorbate. This new approach for generating DNA-CuNPs improves imaging of biological processes and provides a basis for developing other types of DNA-templated nanomaterials.

Keywords DNA-CuNPs · Hydroxyl radical · Biological imaging · Ascorbate · Colon cancer cell · Oxygen · Copper sulfate

Introduction

Biomolecule-directed fluorescent metal nanomaterials have been extensively studied for various applications such as bio-assay and bioimaging [1]. The representative example is the fluorescent DNA-templated copper nanoparticles (DNA-CuNPs), which have been used for biomolecule and cell detection [2–5]. DNA-CuNPs can be prepared in less than 10 min under ambient conditions [6]. In addition, DNA-CuNPs have a long Stokes shift (~300 nm) that is desirable for diagnostic purposes [7–9]. However, the operational lifetime of DNA-CuNPs is fairly short (~20 min), which is a major limitation for bioimaging [10].

Two different strategies have been used to prolong the fluorescence of DNA-CuNPs. One is based on the generation of a long concatemeric DNA by rolling circle amplification, while the other relies on the interaction between DNA and histones [10, 11]. Although these two methods have yielded promising results, they require costly reagents such as DNA polymerase or histones that increase the overall production cost. In addition, the effectiveness of DNA-CuNPs generated by these approaches for bioimaging has not been demonstrated. More seriously, they are only effective for the specific DNA template, limiting the universal application.

In this study, we developed a cost-effective method to improve the fluorescence stability of DNA-CuNPs. We determined that a high concentration of ascorbate promotes the reduction of Cu(II) to Cu(0) and scavenges hydroxyl radical, which is critical for prolonging fluorescence. The fluorescence of DNA-CuNPs generated with optimized condition persisted for 200 min, which is significantly improved compared to 25 min in the conventional synthesis. In addition, we demonstrated the broad applicability of our method by preparing DNA-CuNPs with enhanced fluorescence stability using

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different types of DNA. Finally, we confirmed the utility of DNA-CuNPs for cellular imaging using cultured cells.

Experimental

Materials

All DNA oligonucleotides used in this study were purchased from Integrated DNA Technologies (Skokie, IL, USA, https://sg.idtdna.com/). The oligonucleotide sequences are listed in Table 1. Sodium chloride (NaCl), sodium ascorbate, and sodium sulfite were purchased from Samchun Chemical (Seoul, Korea, http://www.samchun.com/). 3-(N-morpholino) propanesulfonic acid (MOPS) and $CuSO_4$ were from Sigma-Aldrich (St. Louis, MO, USA, https://www.sigmaaldrich.com/). Argon gas was from Green Gas (Seoul, Korea, Phone: +82–2–466-1209). All chemicals were of analytical grade and were used without further purification.

Synthesis of DNA-CuNPs

For the synthesis of DNA-CuNPs by the conventional method, 1 μM DNA and 500 μM $CuSO_4$ were incubated in MOPS buffer (20 mM MOPS and 200 mM NaCl, pH 7.5) for 5 min; 2 mM sodium ascorbate was then added, and the reaction was allowed to proceed for 5 min [2, 3]. To improve the fluorescence stability of DNA-CuNPs, experiments were carried out with varying concentrations of $CuSO_4$ and sodium ascorbate. These concentrations were determined by response surface design using the Minitab program (State College, PA, USA). Except for $CuSO_4$ and sodium ascorbate concentrations, all the conditions were the same as those of the conventional method. Fluorescence signals were measured at an excitation wavelength of 340 nm on a microplate reader (Spectramax iD5; Molecular Devices, Sunnyvale, CA, USA).

Cell culture and nuclear staining for bioimaging

HCT116 (KCLB No. 10247, http://cellbank.snu.ac.kr/) cells were cultured in Dulbecco's modified essential medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA) at 37 °C and 5% CO_2. For cell imaging, 1.6×10^5 cells were seeded in a 35-mm cell culture dish and cultured for 48 h. The cells were fixed and permeabilized using the Fix&Perm Cell Permeabilization Kit (Thermo Fisher Scientific, Waltham, MA, USA). These were then incubated in a solution of 230 μM $CuSO_4$ in MOPS buffer for 10 min; 31 mM sodium ascorbate was then added, followed by incubation for 10 min. In the control group, cells were treated by the conventional method. Except for $CuSO_4$ (500 μM) and sodium ascorbate (2 mM) concentrations, all of the conditions were the same as those in the new synthesis method. The stained cells were imaged under a fluorescence microscope (BX51, Olympus, Tokyo, Japan) with excitation (330–385 nm) and barrier (420 nm) filters.

Results and discussion

Stability of DNA-CuNPs

Figure 1 shows the mechanism for the poor stability of DNA-CuNPs generated by the conventional method. Oxygen (O_2) oxidizes Cu(0) to Cu(II) (i) while producing •OH (ii) under

Table 1 DNA sequences used in this study

Name	Sequence	References
S1 (Single stranded DNA)	5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′	[12]
S2 (Hairpin DNA)	5′-CGCCGCCGGCGCGCGCCGC 3′-GCGGCGGCCGCGCGCGGCG TTTTTTTTTTTTTTTTTTTTTTTTTTTTT	[13]
S3 (Double stranded DNA)	5′-ATATATATATATATATATATATATATATAT-3′ 3′-TATATATATATATATATATATATATATATA-5′	[14–16]

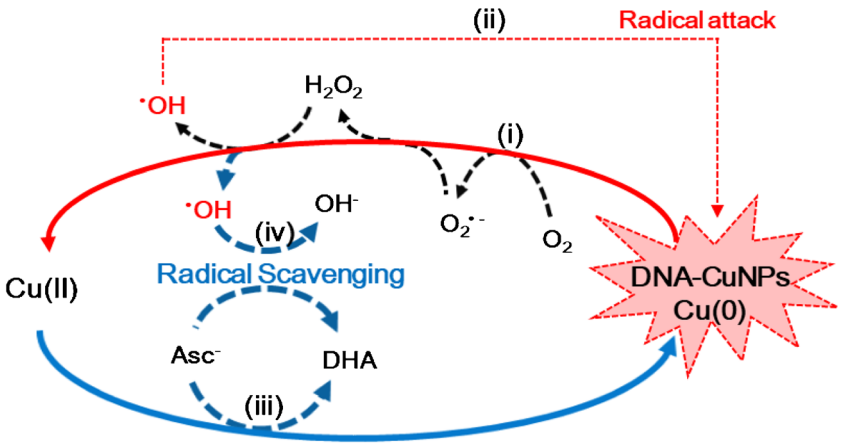

Fig. 1 Mechanism for the poor stability of DNA-CuNPs synthesized by conventional condition. Under ambient oxygen (O_2)-rich conditions, Cu(0) of DNA-CuNPs is oxidized to Cu(II) (i) and hydroxyl radical (•OH) is produced, which attacks DNA-CuNPs (ii). Ascorbate (Asc^-) reduces Cu(II) to Cu(0) of DNA-CuNPs (iii) and scavenges OH^- (iv). DHA, dehydroascorbic acid

ambient conditions [17, 18]. We speculated that DNA-CuNPs formed on the DNA template by the reduction of Cu(II) to Cu(0) in the presence of ascorbate (iii). Then they would quickly lose their fluorescence in an oxygen-rich environment that promotes reactions (i) and (ii) [19]. To test this hypothesis, we prepared an oxygen-deficient environment for the formation of DNA-CuNPs either by argon purging or by treatment with sodium sulfite, an oxygen scavenger. In the absence of oxygen (Fig. S1), the fluorescence emission of DNA-CuNPs was more stable than under oxygen-rich conditions. We then investigated whether reactions (i) and (ii) are the major contributors to the decreased fluorescence intensity of DNA-CuNPs. We first tested reaction (i) by applying ascorbate immediately after the disappearance of the fluorescence signal. Fluorescence was restored by adding ascorbate (Fig. S2(A)), confirming reaction (i)—i.e., the oxidization of Cu(0) to Cu(II) in the presence of oxygen. We next carried out gel electrophoresis to evaluate reaction (ii), in which the DNA template is attacked by •OH. The DNA template was damaged when the fluorescence signal of DNA-CuNPs was abolished, as evidenced by the weakened band intensity (Fig. S2(B)). These results suggest that factors that reduce Cu(II) to Cu(0) (iii) and scavenge •OH (iv) mitigate the deleterious effects of oxygen (i and ii). As a result, DNA-CuNPs' fluorescence stability is increased (Fig. 1).

Synthesis of DNA-CuNPs with stable fluorescence

To improve the fluorescence stability of DNA-CuNPs, we first investigated the effects of sodium ascorbate and copper sulfate ($CuSO_4$). These two factors were selected because these are the only factors involved in the redox reaction that generates DNA-CuNPs. For quantitative analysis of fluorescence stability, we defined quarter time (T_Q) as the time to reach one quarter of the initial fluorescence intensity. The following parameters were optimized: (a) Concentration of ascorbate; (b) concentration of $CuSO_4$. Respective text and Figures on optimizations are given in the Electronic Supporting Material (Fig. S3 and Fig. S4). In short, the following experimental conditions were found to give best results: (a) Best concentration of ascorbate: 31 mM; (b) Best concentration of $CuSO_4$: 230 μM. The maximum T_Q (161 min) and initial fluorescence intensity (2.16×10^6) were obtained at these conditions (Fig. S4).

Next, we compared the new synthetic condition with the conventional one. For this, the relative fluorescence intensity

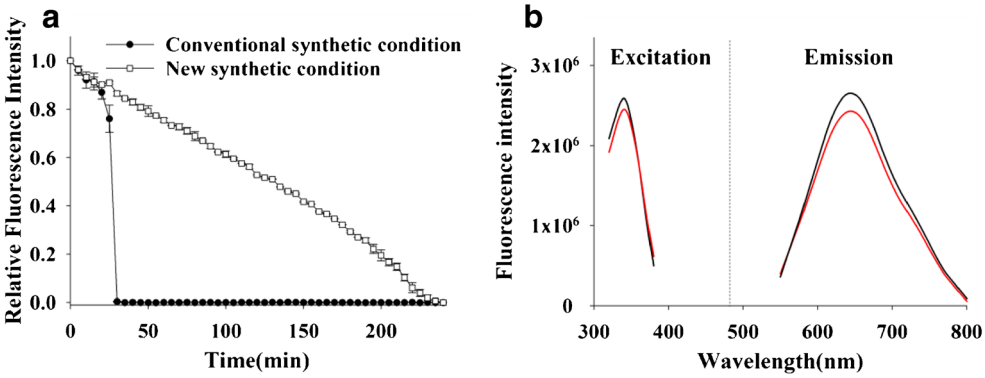

Fig. 2 Comparison of fluorescence stability (**a**) and intensity (**b**) of DNA-CuNPs generated by the conventional and new synthetic approaches. The first method (black) uses 500 μM $CuSO_4$ and 2 mM sodium ascorbate, whereas our method (red) uses 230 μM $CuSO_4$ and 31 mM sodium ascorbate. In both cases, the concentration of S1 DNA template was 1 μM

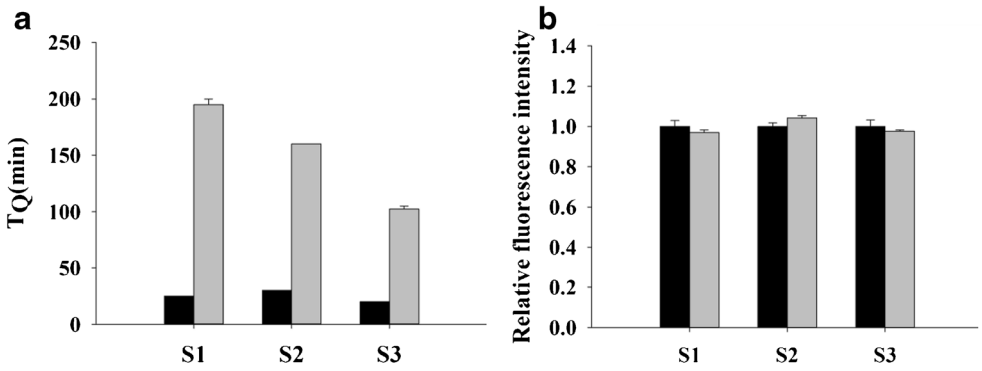

Fig. 3 **a** Fluorescence stability and (**b**) intensity of DNA-CuNPs generated using S1, S2, and S3 DNA templates (1 μM). The conventional synthesis method (black) uses 500 μM $CuSO_4$ and 2 mM sodium ascorbate, whereas the new synthetic method (gray) uses 230 μM $CuSO_4$ and 31 mM sodium ascorbate

($F \cdot F_0^{-1}$), which was calculated by dividing the fluorescence intensity (F) at a given time point by F_0, the fluorescence intensity at 0 min. The results in Fig. 2a show that the fluorescence of CuNPs in the conventional method rapidly declines to 0 after just 20 min. In contrast, for DNA-CuNPs prepared by the new method, the fluorescence persisted for more than 200 min (Fig. 2a) and F_0 at both excitation and emission wavelengths was maintained (Fig. 2b).

Universal applicability of DNA-CuNPs synthesized by the new method

The universal applicability of our method was evaluated using different types of template DNA. We included single-stranded DNA (S1), hairpin DNA (S2), and double-stranded DNA (S3), which are known to form DNA-CuNPs. The T_Q of S1, S2, and S3 increased to 7.8, 5.3, and 5.1 times compared with the values under the conventional synthesis method (Fig. 3a), confirming the broad applicability of our strategy. It should be noted that F_0 under the new synthesis conditions was compared to the values under the conventional method (Fig. 3b).

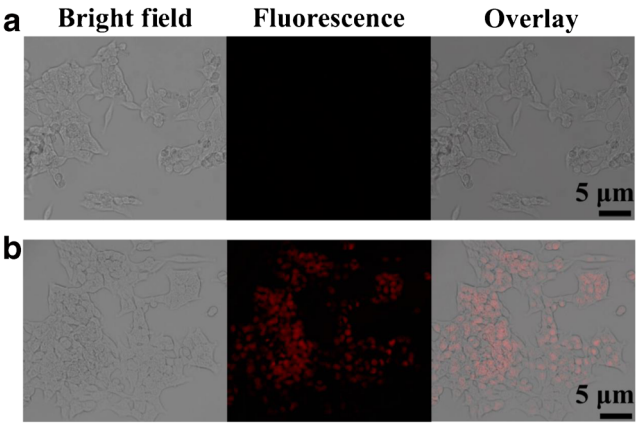

Fig. 4 Fluorescence micrographs of HCT116 cells stained with DNA-CuNPs. **a**, **b** Cells stained with DNA-CuNPs generated by the conventional method (**a**) and the new method (**b**)

Application of DNA-CuNPs to cell imaging

We examined the feasibility of using DNA-CuNPs with enhanced fluorescence stability for cell imaging. As a proof-of-concept, we stained the nucleus, which is packed with double-stranded, genomic DNAs of random sequence known to form DNA-CuNPs with the less efficiency [14]. HCT116 human colon cancer cells were fixed, permeabilized, and treated with the new method. Cell nuclei were successfully stained with DNA-CuNPs, showing a bright red fluorescence signal (Fig. 4). Importantly, control cells treated with the conventional method did not exhibit any fluorescence.

Conclusion

We developed a low-cost approach for synthesizing DNA-CuNPs with a longer operational lifetime. We determined the molecular basis for the enhanced stability of DNA-CuNPs and the optimal conditions for their generation. We also confirmed that the new synthesis method is applicable to different types of DNA template, and used the DNA-CuNPs with more stable fluorescence to stain cell nuclei for imaging. This novel method not only expands the scope of application of DNA-CuNPs but also provides an efficient and cost-effective strategy for developing other fluorescent nanomaterials.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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