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Near-Infrared Photothermal Activated DNazymes-Gold Nanoshells for Imaging Metal Ions in Living Cells

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Abstract

DNazymes have enjoyed success as metal ion sensors outside cells. Their susceptibility to metal-dependent cleavage during delivery into cells has limited their intracellular applications. To overcome this limitation, we herein report a near-infrared (NIR) photothermal activation method for controlling DNzyme activity in living cells. The system consists of a three-stranded DNzyme precursor (TSDP) whose hybridization prevents the DNzyme from being active. After conjugating the TSDP onto gold nanoshells and upon NIR illumination, the increased temperature dehybridizes the TSDP to release the active DNzyme, which then carries out metal ion-dependent cleavage, resulting in releasing the cleaved product containing a fluorophore. Using this construct, detecting Zn²⁺ in living HeLa cells is demonstrated. This method has expanded the DNzyme versatility for detecting metal ions in biological systems under NIR light that exhibits lower phototoxicity and higher tissue penetration ability.

TOC image

Near-IR activated DNzyme sensors: A near-IR light activated DNazymes-AuNS system has been developed and applied for imaging metal ions in living cells.

Keywords

DNAzymes; Metal ions; Gold nanoshells; Photothermal activation; HeLa cells

Metal ions are vital components in biological systems, where they play important roles.^[1] The disruptions in metal homeostasis are implicated in many severe diseases.^[2] In order to understand the metal homeostasis in human health and disease, probes that can identify specific metal ion species and their subcellular locations are of significant interests.^[3] Toward this goal, many fluorescent probes based on either small organic molecules or fluorescent proteins have been developed.^[4] Despite numerous groups' efforts for many years, there are only a limited number of metal ion sensors that were effective in sensing metal ions in cells, even though the large number of metal ions in the biological system demands a more generalizable approach to develop sensors for as many metal ions as possible. To meet such a demand, we and others have taken advantage of DNAzymes or deoxyribozymes, which are DNA molecules capable of performing enzymatic reactions in presence of metal cofactors.^[5] In this complementary approach to rational design of fluorescent sensors, metal-specific DNAzymes can be obtained by a combinatorial process called *in vitro* selection starting from a large DNA library of up to 10^{15} combinations.^[6] In this way, it is not necessary to have advanced knowledge to construct metal-binding site. The selection process allows the binding affinity and selectivity of the DNAzymes to be fine-tuned by introducing different levels of stringency. As a result, many DNAzymes with high selectivity have been obtained^[5b,7] and then converted into metal sensors.^[8] These sensors are playing key roles in environmental monitoring, including monitoring Pb^{2+} in municipal water system in public schools in the US.^[9]

In contrast to much progress made in using DNAzymes to monitor metal ions in the environment, applications of DNAzyme in imaging of metal ions in living cells have begun to appear only recently.^[10] A major barrier to the progress is that the DNAzymes can react with extracellular metal ions and cleave its substrate during the delivery process, before they reach the desired locations in cells. To overcome this barrier, we have developed a photochemical caging strategy to render the DNAzymes inactive during the delivery progress, and then activate the DNAzyme by UV light once the DNAzymes are inside the cells.^[11] While this strategy has been demonstrated to work in living cells, it requires high-energy UV irradiation that can harm the cells. Therefore, it is highly desirable to develop methods to control DNAzymes activity without damaging the cells. We herein report a novel

method of near-infrared (NIR) photothermal activated DNAzyme conjugated to gold nanoshells (AuNS) for detection of metal ions in living cells.

We choose to use NIR light to control DNAzymes activity in living cells, because it exhibits lower photo-toxicity while providing higher tissue penetration ability than UV light.^[12] AuNS are known to absorb NIR light and efficiently convert it into heat due to the large absorption cross-sectional inability to emit light.^[13] This photothermal property of AuNS has been used in targeted drug delivery and cancer therapy.^[14] One particular interesting feature of AuNS is that the NIR irradiation can release double stranded DNA (dsDNA) from AuNS by increasing the local temperature above the melting temperature of the dsDNA.^[15] Taking advantage of these properties, we designed a three-stranded DNAzyme precursor (TSDP) consisting of an enzyme strand with a black hole quencher (BHQ2) at the 3'-end that can hybridize to a substrate strand with Cy5 at the 5'-end on the right arm (in orange, Scheme 1a), and to a Linker DNA strand with -SH group at the 5'-end on the left arm (in purple). The TSDP can be conjugated onto AuNS through thiol-gold chemistry. At 37 °C where the cells are cultured, the hybridization of the Linker DNA strand (in blue) with the left arm of the enzyme strand, which is designed to display a calculated melting temperature around 50 °C, prevents the enzyme strand from hybridizing to the left arm of the substrate strand and thus the DNAzyme from being active.

Since Cy5 is close to AuNS, the Cy5 fluorescence is thereby quenched. Upon NIR irradiation, the local temperature around AuNS increased to above the melting temperature between the Linker strand and the enzyme strand, which causes a release the enzyme and substrate strands from AuNS. Once free of the Linker strand, the left arm of the enzyme strand can hybridize with the substrate strand to form an active DNAzyme. In this configuration, since Cy5 is next to BHQ2, the Cy5 fluorescence is quenched by BHQ2. In the presence of Zn^{2+} , the substrate strand will be cleaved. Because the calculated melting temperature between the left arm of the DNAzyme and the cleaved product is around 20 °C, the cleaved product containing Cy5 will be released at 37 °C from the enzyme strand and away from BHQ2, resulting in an increased fluorescent signal.

The TSDP was prepared by heating and annealing the -SH modified Linker DNA (Lane 1, Figure 1) with the enzyme strand (Lane 2), resulting in a band (Lane 4) with higher molecular weight (MW) consistent with hybridization between the two strands. The thiolated DNA contains a poly T spacer to avoid the steric interference of the AuNS to DNA hybridization. The construct was then annealed with the substrate strand (Lane 3), resulting in a strong top band (Lane 6) with MW similar to that of the TSDP and a minor lower band consistent with hybridization of enzyme and substrate strand, like in Lane 5. In the presence of Zn^{2+} , the enzyme/substrate strand in Lane 5 displayed a lower MW band (Lane 7), suggesting that the substrate band was cleaved. In contrast, the top band, assigned to TSDP in Lane 6 remained the same in the presence of Zn^{2+} (Lane 8), indicating that, even in the presence of Zn^{2+} , the DNAzyme is blocked from being active. To test the long-term stability of the TSDP, the construct was incubated for 24 h in the presence of Zn^{2+} . The TSDP remained the same with little degradation (Figure S1), suggesting that this TSDP is “caged” without being cleaved.

Having demonstrated successful preparation of the TSDP, we synthesized the AuNS according to the reported protocol.¹³ As the transmission electron microscopy (TEM, Figure 2a) and scanning electron microscopy (SEM) (Figure S2) images shown, the size of AuNS is ~150 nm. Illuminating the AuNS with 808 nm laser (1 W/cm²) for 10 min caused an increase in temperature that depended on the particle concentration (Figure 2b). The TSDP was conjugated onto AuNS using the modified salt-aging method.¹⁶ The TSDP-functionalized AuNS (called TSDP-AuNS hereafter) are similar in the shape and dispersity to those of bare AuNS (Figure 2c). While suspending bare AuNS in the buffer containing 100 mM NaCl caused them to aggregate in several minutes, as indicated by the broad optical extinction spectrum (Figure 2d, blue line), redispersion of TSDP-AuNS in buffer caused minimal change to the UV spectrum, suggesting that the stability of the AuNS was significantly enhanced after the TSDP functionalization. The number of TSDP on each AuNS was calculated to be 3635±534 using fluorescence method (Figure S3).^[13,15] In addition, the Cy5 fluorescence of TSDP-AuNS was completely quenched (Figure S4).

After conjugating the TSDP onto AuNS, we evaluated the feasibility of TSDP-AuNS as a biosensor in a buffer (50 mM Tris, 100 mM NaCl, pH 7.4). As shown in Figure 3a, the TSDP-AuNS showed minimum fluorescence, indicating Cy5 has been nearly completely quenched by AuNS. The construct was irradiated for 15 min. using a laser with a power density of 1 W/cm², which is commonly used for cellular study.^[15b] After NIR irradiation, in the absence of Zn²⁺, the construct displayed an enhanced fluorescence signal around 660 nm, consistent with the DNAzyme being released from AuNS, as shown in Scheme 1a. Since BHQ2 is a poorer quencher compared with AuNS, the fluorescence of Cy5 was recovered. Upon addition of Zn²⁺ into the supernatant, the fluorescence was enhanced further by nearly 4 folds, demonstrating that cleaved product containing Cy5 has been released from the enzyme strand containing BHQ2. To support the above findings, we further used polyacrylamide gel electrophoresis (PAGE). As shown in Figure 3b, without NIR irradiation or addition of Zn²⁺, no DNA band was observed on the supernatant (Lane 1 and 4), suggesting that the DNAzymes stayed on the AuNS. NIR irradiation resulted in observation of a DNA band with MW consistent with that of the active DNAzymes (Lane 5). A band with a similar MW was observed when the same system is treated with a hot water bath (Lane 6), suggesting that the NIR photothermal decaging has the same effect as thermal decaging in releasing the active DNAzymes. Upon addition of Zn²⁺, the above NIR irradiated or thermally decaged constructs showed an even lower band (see Lane 2 and 3, respectively) whose MW is similar to the cleaved products. Together, these results indicate successful controlled NIR photothermal caging and decaging of the TSDP-AuNS. The response of released DNAzymes to different concentrations of Zn²⁺ was tested (Figure S5), which showed similar kinetics to free DNAzymes.

To achieve the best performance of the biosensor, we optimized the length of the Linker DNA (Figure S6). While the release efficiencies were very similar, the SH-16 sequence consistently shows higher release efficiency and thus chosen for further studies. Before we explored TSDP-AuNS for cellular studies, we tested its stability and feasibility in cell lysate (Figure S7). After a 7 h incubation, there was no fluorescence signal increase, indicating that the construct is stable in cell lysate. After NIR irradiation, adding Zn²⁺ enhanced the

fluorescence by 2.4 folds, which is similar to the result observed in the buffer, suggesting TSDP-AuNS can be used as a biosensor in cells.

Finally, we tested the performance of TSDP-AuNS for sensing Zn^{2+} in HeLa cells. The probe was incubated with the cells for 6 h to allow sufficient uptake. Upon NIR irradiation, followed by the incubation of Zn^{2+} , confocal microscopy was used to compare samples with and without NIR irradiation and with and without Zn^{2+} incubation. As shown in Figure 4, without NIR irradiation and even with addition of Zn^{2+} , there was minimal fluorescent signal in the Cy5 channel, indicating successful photothermal caging of the DNAzymes. After NIR irradiation, but without addition of Zn^{2+} , some fluorescence of Cy5 was recovered, and this recovery may come from the released DNAzymes that reacted with endogenous mobile Zn^{2+} . To further corroborate this finding, we added 10 μM Zn^{2+} into the cells after NIR irradiation, and observed dramatic increase of the fluorescent intensity. A colocalization study (Figure S8) indicated that the fluorescence comes mainly from cytoplasm, suggesting that the AuNS can escape from lysosomes into cytoplasm (for discussion of mechanisms, please see legend of S8), making this biosensor system an excellent choice for imaging metal ions in different compartments of the cell.

To make sure that the fluorescent signal indeed comes from the catalytic activity of the DNAzyme, instead of intracellular DNA or RNA nucleases-mediated substrate cleavage, we employed either a non-cleavable substrate or an inactive mutant DNAzyme as negative controls (Figure S9). Under the same condition, neither of two negative controls samples gave visible fluorescent signals, while the active DNAzyme with cleavable substrate showed enhanced fluorescent signal, demonstrating that the fluorescent signal came from metal-ion specific catalytic activity of the DNAzyme. To confirm that the observations from the above confocal images apply to the whole cell population, flow cytometry was employed (Figure S10). HeLa cells treated with both NIR irradiation and Zn^{2+} had a higher level of fluorescence intensity than those with either no NIR irradiation or no Zn^{2+} addition. Together, these results indicate that TSDP-AuNS can be employed to image metal ions in living cells.

In conclusion, we have developed a NIR light activated DNAzymes-AuNS system, and demonstrated its application in detecting metal ions in living cells. During their delivery into cells, the activity of DNAzymes could be inhibited, protecting the substrate strand from being cleaved by extracellular metal ions. The AuNS allows NIR light-activated photothermal dehybridization, which activates the DNAzymes for metal-dependent cleavage and release of products with fluorophore attached. This NIR activated DNAzyme-based sensing system broadens the range of applications of DNAzymes in living cells, making it a powerful tool for cellular imaging applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Native PAGE of TSDP formation and stability in the presence of metal ion. The gel was stained with ethidium bromide for visualization. M: DNA ladder; Lane 1: Linker DNA (the ethidium bromide does not stain short single stranded Linker DNA); Lane 2: Enzyme strand; Lane 3: Substrate strand; Lane 4: Linker DNA+ Enzyme strand; Lane 5: Enzyme strand+ Substrate strand; Lane 6: Linker DNA+ Enzyme strand+ Substrate strand; Lane 7: lane 5+ Zn^{2+} ; Lane 8: lane 6+ Zn^{2+} . The dotted lines (from top to bottom) indicate TSDP, DNAzymes and cleaved DNAzymes.

Figure 2. Characterization of AuNS and TSDP-AuNS. a) TEM image of bare AuNS; b). Photothermal response of bare AuNS at different concentrations.; c) TEM image of TSDP-AuNS; d). Absorption spectra of bare AuNS in water (black line), bare AuNS in buffer (blue line) and TSDP-AuNS in buffer (red line). Scale bar: 200 nm.

Figure 3.

DNAzyme release study from TSDP-AuNS under NIR irradiation. a). Fluorescence spectra of TSDP-AuNS before NIR irradiation and supernatant of TSDP-AuNS after NIR irradiation under different conditions; b). 12% PAGE. M: DNA ladder; Lane 1 and 4: TSDP-AuNS without NIR irradiation or addition of Zn^{2+} ; Lane 2: NIR irradiation for 15 min, with Zn^{2+} ; Lane 3: 70 °C water bath for 15 min, with Zn^{2+} ; Lane 5: NIR irradiation for 15 min, without Zn^{2+} ; Lane 6: 70 °C water bath for 15 min, without Zn^{2+} . The dotted lines (from top to bottom) indicate released active DNAzymes and cleaved DNAzymes.

Figure 4.
Confocal microscopy images of HeLa cells. Scale bar: 20 μ m.

Scheme 1.

Design of NIR activated TSDP-AuNS for intracellular metal ion sensing.