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DNAzyme crosslinked hydrogel: a new platform for visual detection of metal ions

A DNAzyme crosslinked hydrogel capable of undergoing catalytic gel-sol transition in response to copper ions was developed. The smart hydrogel was demonstrated to be an excellent platform for visual detection of metal ions, and can possibly be used as target-responsive drug delivery systems and other environmentally responsive materials.

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DNAzyme crosslinked hydrogel: a new platform for visual detection of metal ions†

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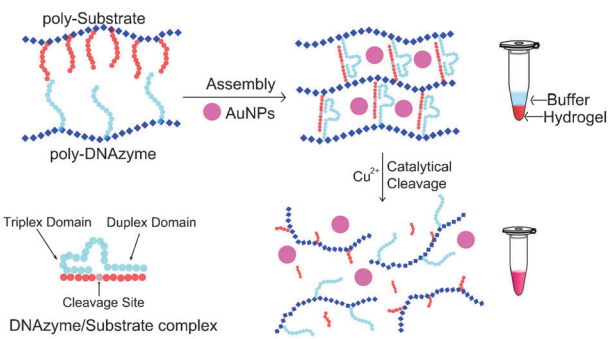
We propose the use of DNAzyme as a crosslinker of hydrogel to develop a catalytic platform for the sensing of metal ions. The DNAzyme crosslinked hydrogel can undergo gel–sol transition in response to Cu^{2+} ions, which enables sensitive visual detection of Cu^{2+} by observing the release of pre-trapped AuNPs.

Hydrogels are soft materials with a crosslinked 3D network structure that contains a large fraction of water within their structure. In a hydrogel, the backbone molecules are held together by crosslinkers which keep the chains in the polymer matrix together, preventing the dissolution of the long molecular chains and increasing the mechanical stability of the hydrogel. Because of their biocompatibility, flexibility and mechanical stability, hydrogels have many potential applications, particularly in biotechnological and biomedical fields. Among the many types of hydrogels, stimuli-responsive hydrogels, which undergo volume transitions in response to physical or chemical changes, have been of great interest. These intelligent materials may be suitable for bioseparation, biosensing, and drug delivery.[1] To date, various hydrogel sensors that are responsive to stimuli, such as changes in temperature, pH, ion concentration, light, and electric field, have been reported.[2] Most of these sensors are based on mechanical work performed by the gel swelling and shrinking or on changes in the properties of the free swelling/shrinking gels, such as optical transmission,[3] refractive index,[4] or resonance frequency.[5] More recently, several bioresponsive hydrogels have been reported where biomolecular recognition pairs including antibody–antigen,[6] DNA/DNA, DNA/Aptamer[7] have been used as crosslinkers. As a result, binding competition of free target molecules with the crosslinking pair will reduce the crosslinking ratio, eventually leading to swelling or breaking of hydrogel. Based on this working principle, many elegant target-responsive drug delivery systems and biosensing methods have been reported. For example, we have designed a DNA/Aptamer crosslinking hydrogel for highly sensitive cocaine visual detection.[7g] Theoretically, in these bioresponsive hydrogels, one target molecule will only break one crosslinking pair, which might limit the response or sensitivity of the hydrogel. The sensitivity of the hydrogel could be improved by designing a hydrogel in which a target molecule can catalytically break a large amount of crosslinkers. Herein, we report a new target responsive DNAzyme crosslinked DNA hydrogel based on catalytically site-specific self-cleavage of the crosslinkers, which sidesteps the mechanism of stoichiometric competition.

DNAzymes are DNA molecules with catalytic activity which can be acquired *via in vitro* selection.[8] Since the first discovery of DNAzymes, it has been found that they can catalyze reactions of ligation, DNA/RNA self-cleavage, *etc.*[9] In most DNA/RNA self-cleavage reactions, metal cations are necessary cofactors. Metal ions can specifically recognize the DNAzyme secondary structure, and then irreversibly cleave DNA/RNA at the cleavage site. The indispensability of metal ions provides a novel access to detection of metal ions.[10] Lu and other workers have elegantly tailored DNAzymes into many highly sensitive and selective metal sensors, such as Pb^{2+},[11] Hg^{2+},[12] Cu^{2+},[13] UO_2^{2+}.[14] Inspired by the exceptional performance of DNAzyme sensors, we proposed the use of DNAzymes as a crosslinker of hydrogel to develop a catalytic platform for metal ion sensing.

To prove the concept, we chose the Cu^{2+} DNAzyme sequence reported by Liu and Lu as a model.[13] The principle of our work is illustrated in Scheme 1. The DNAzyme complex consists of two strands of DNA, DNAzyme and Substrate.



Scheme 1 The working principle of copper ion responsive DNAzyme crosslinked hydrogel.

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The DNAzyme and Substrate are incorporated into linear polyacrylamide, respectively, to form DNA side-chain polymers (poly-DNAzyme and poly-Substrate). When the polymers are mixed together, they crosslink to form a 3D network by hybridization between the DNAzyme and Substrate, and the rigid DNAzyme/Substrate complex transforms the linear polymer solution into a highly crosslinked hydrogel.

The DNAzyme/substrate complex contains a duplex domain and a triplex domain. Upon the addition of Cu^{2+}, the substrate is irreversibly cleaved at the cleavage site that creates a less stable triplex crosslinker which subsequently leads to the dissociation of the two DNA side-chain polymers and eventually dissolution of hydrogel into the liquid form.

The rigid spacer formed by the DNAzyme complex also provides pores to encapsulate and release molecules. In order to visually detect the change of DNAzyme hydrogel without any advanced or complicated instruments, gold nanoparticles (AuNPs) are introduced in our system. AuNPs can be well dispersed in solution, and have remarkable absorption in the visible spectrum (around 520 nm). In this system, AuNPs serve as an indicator for gel–sol transformation. When AuNPs are added with the two DNA side-chain hybridizing polymers, AuNPs are trapped in the DNAzyme hydrogel pores. However, after introducing Cu^{2+}, AuNPs will be gradually released into the buffer along with dissolution of hydrogel. This procedure can be visually observed and forms the basis of our visual detection method.

In order to obtain a stable hydrogel, we first optimized the DNAzyme/Substrate pair. Since the DNAzyme hydrogel is held together by the hybridization of the DNAzyme and Substrate sequences, the stability of the hydrogel is affected by the affinity of the DNAzyme/Substrate complex. The higher the binding affinity, the more stable a hydrogel will form. On the other hand, after cleavage the hydrogel is crosslinked by the triplex domain of the DNAzyme/Substrate and its stability is mainly affected by the affinity of the triplex. To obtain a stable hydrogel before cleavage at room temperature, we could increase the binding affinity of DNAzyme to its substrate by extending the length of the duplex domain of the DNAzyme/Substrate pairs whilst carefully maintaining its catalytic property. To investigate the feasibility, we designed three pairs of DNAzyme/Substrate with different duplex domains, Enyzme35/Substrate24, Enyzme37/Substrate26, Enyzme39/Substrate28 (see ESI†). The Enzyme35/Substrate24 was previously used.[13] Melting analysis results indicated that this pair only has a melting temperature (T_m) of 30 °C. Hydrogel with Enzyme35/Substrate24 as crosslinker would not be stable at room temperature. By extending two base pairs of the duplex domain, Enzyme37/Substrate26 was found to have a T_m of 35 °C. We further increased the length of the duplex domain by 2 more bases and the resulting Enzyme39/Substrate28 was found to have a T_m of 43 °C (Fig. S1, ESI†). The cleavage efficiency of all enzymes was investigated. Interestingly, our results suggested that the cleavage efficiency of the DNAzyme increased with the extension of the duplex domain. For example, the relative enzymatic activity of Enzyme39/Substrate28 was as high as 6 times that of the Enzyme35/Substrate24 (Fig. S2, ESI†). Such a significant improvement in enzyme activity was likely due to efficient binding of the enzyme to the substrate as a result of increase in binding affinity.[15] As a result, we chose the Enyzme39/Substrate28 pair as the crosslinker of our hydrogel.

The DNAzyme and substrate were grafted onto a linear polyacrylamide chain *via* free-radical polymerization to form poly-Substrate and poly-DNAzyme, respectively (see ESI†). Poly-Substrate and poly-DNAzyme were then mixed with BSA coated 13 nm AuNPs to form a homogeneous red-colored hydrogel by forming a DNAzyme/Substrate complex. When buffer solution was added to the gel, an apparent boundary between gel and supernatant could easily be observed with the naked eye, suggesting the successful formation of hydrogel at room temperature. However, upon addition of Cu^{2+}, the gel dissolved and the AuNPs diffused into the upper layer. To confirm that the dissolution of hydrogel was indeed the result of copper initiated enzymatic cleavage of the substrate, we replaced the DNAzyme sequence in the hydrogel with a sequence that is completely complementary to the substrate as a comparison. This pair of complementary DNA could also form a DNA hydrogel by direct hybridization. An apparent boundary between the mutated gel and supernatant could also be observed. However, since the mutated DNA had no enzymatic activity even in the presence of Cu^{2+}, the mutated gel remained unchanged with the addition of Cu^{2+} (Fig. 1a), which shows the discrimination between mutated DNA hydrogel and DNAzyme hydrogel.

The kinetics of copper ion catalyzed hydrogel dissolution was studied by monitoring the release of the trapped AuNPs (Fig. 1b). The release of AuNPs was monitored by UV/Vis spectroscopy at 520 nm every 20 min. The UV/Vis absorption spectrum indicated that as time elapsed, AuNPs gradually diffused into the supernatant with disruption of the gel, and the whole gel vanished after around 1.5 h with 100 μM Cu^{2+}. In contrast, no obvious absorbance change for the control sample was observed. The Cu^{2+} concentration also had an impact on dissolution kinetics. Hydrogel treated with 5 μM Cu^{2+} could be distinguished from gel without copper ions after overnight incubation. However, it only took 20 min to dissolve the gel with 10 mM Cu^{2+}. This could assist us to semi-quantify different concentrations of Cu^{2+}. Theoretically, one copper ion can initiate the cleavage of many

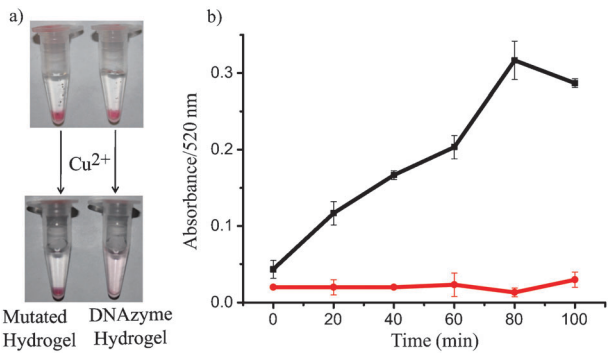

Fig. 1 Response of DNAzyme hydrogel to copper ions (a) and kinetic study of gel dissolution (b). The concentration of copper ions in the experiment was 100 μM. Black line: time course of AuNPs release after addition of 100 μM Cu^{2+}. Red line: time course of AuNPs release when no Cu^{2+} was added.

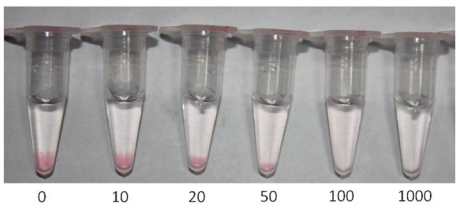

Fig. 2 Visual detection of different concentrations of copper ions. Each tube contains 9 μL gel and 100 μL buffer solution, and the reaction time was about 1.5 h.

DNAzyme/substrate pairs. As a result, a small amount of copper ions could lead to the complete breakdown of hydrogel. To evaluate the sensitivity of our hydrogel assay, the response of hydrogel to different concentrations of copper ions was investigated. As shown in Fig. 2, the size change of the hydrogel is proportional to the copper concentration. Within 1.5 hour, 100 μM Cu^{2+} led to the complete dissolution of the gel. With the same incubation time, only half of the gel disappeared with 20 μM Cu^{2+}. At a 10 μM Cu^{2+} concentration level, only about 10% of the hydrogel disappeared. It is worth noting that the US Environmental Protection Agency's upper limit for copper in drinking water is 20 μM. The results suggest that our hydrogel has great potential for highly sensitive and rapid detection of copper ions in drinking water.

To investigate the selectivity of DNAzyme-Hydrogel, other metals that widely exist in drinking water were used in place of Cu^{2+} with 10 fold higher concentrations. As shown in Fig. 3, among the tested metals (Cu^{2+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, Hg^{2+}, Pb^{2+}, Cd^{2+}), only Cu^{2+} resulted in the disruption of the DNAzyme-Hydrogel and generated a clear response.

In conclusion, we have developed a new, simple platform for the visual detection of copper ions through a copper dependent DNAzyme crosslinked hydrogel. Based on the specific recognition of the DNAzyme, the crosslinked structure could be catalytically destroyed in the presence of Cu^{2+}. We were able to use this simple system to detect less than 10 μM Cu^{2+} with the naked eye within 1.5 h, without the aid of any sophisticated instruments. Our technique can be easily applied to the detection of other metal ions, such as Pb^{2+}, UO^{2+}, Cr^{3+}, Zn^{2+} and Mn^{2+} by using the corresponding DNAzymes.

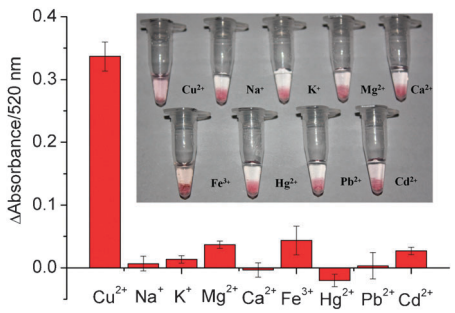

Fig. 3 The selectivity of the DNAzyme hydrogel system. The concentration of Cu^{2+} was 100 μM while other metal ions were all 1 mM, and the reaction time was about 1.5 h.

Combining these advantages, metal ion responsive hydrogels will find wide application in the simple and rapid detection of metals for environmental monitoring with excellent selectivity and high sensitivity.

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