A DNAzyme cascade for the amplified detection of Pb^{2+} ions or L-histidine†

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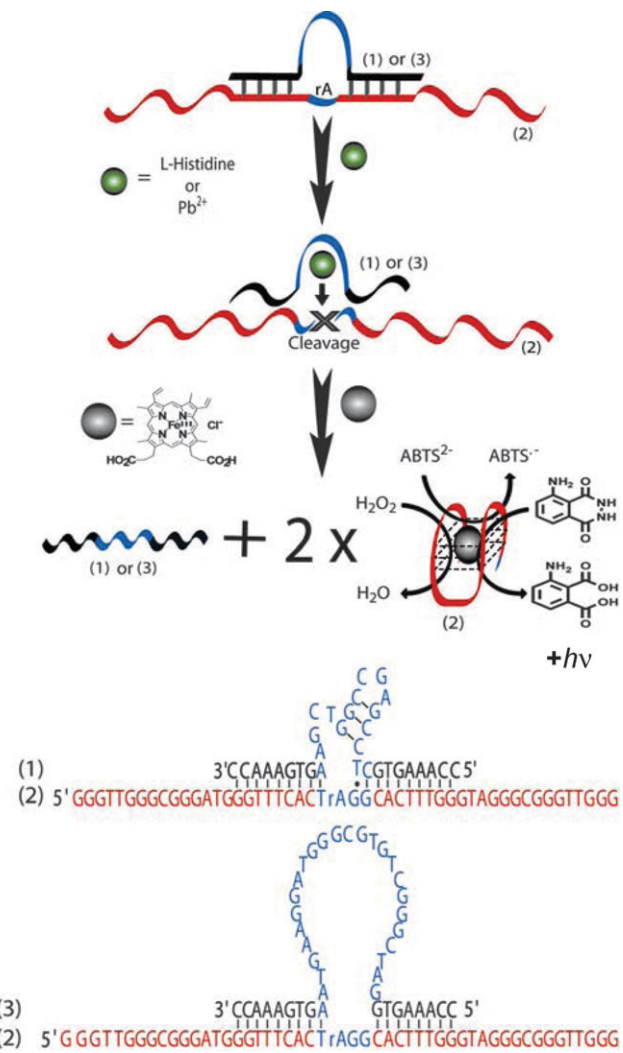
DNAzyme cascades activated by Pb^{2+}- or L-histidine-dependent DNAzymes yield the horseradish peroxidase-mimicking catalytic nucleic acids that enable the colorimetric or chemiluminescence detection of Pb^{2+} or L-histidine.

Catalytic nucleic acids, DNAzymes or ribozymes attract growing interest as catalytic labels for sensing events.[1] For example, a Pb^{2+}-dependent DNAzyme was used to develop optical Pb^{2+} sensors by the activation of fluorescence though the cleavage of a fluorophore-quencher-modified substrate by the DNAzyme[2] or by the deaggregation of Au nanoparticles by the DNAzyme scission of nanoparticle-bridged nucleic acid aggregates.[3] Similarly, a Pb^{2+} electrochemical sensor was developed by the tethering of a redox-active group to the DNAzyme and the cleavage of the enzyme substrate on an electrode surface.[4] A horseradish peroxidase-mimicking DNAzyme, consisting of hemin intercalated in a G-quadruplex structure,[5] was extensively studied as a biocatalytic label for the amplified biosensing of nucleic acids or telomerase activity.[6] Also, its generation by DNA-based machines, (*e.g.*, replication/scission or rolling circle amplification) activated by a recognition process, provided the DNAzyme that generated colorimetric or chemiluminescence readout signals.[7,8]

Recently, the horseradish peroxidase-mimicking DNAzyme was conjugated to aptamers, and the formation of the aptamer–analyte complex released the DNAzyme that acted as a biocatalyst for the amplified readout of the recognition event.[9] Here we wish to report on a general method to use DNAzyme cascades for amplified sensing, and we exemplify the approach by analyzing Pb^{2+} ions or L-histidine.

Scheme 1 depicts the method to design the DNAzyme cascade system for analyzing Pb^{2+}. The nucleic acid (**1**) includes the sequence that organizes to the Pb^{2+}-dependent cleaving DNAzyme. To the ends of the DNAzyme two nucleic acid sequences partially complementary to the horseradish peroxidase-mimicking DNAzyme[5] are tethered. The substrate of this DNAzyme, (**2**), includes the base sequence with the specific ribonucleobase that is cleaved by the Pb^{2+}-DNAzyme. The substrate oligonucleotide is tethered at the 3′ and 5′ ends to the horseradish peroxidase (HRP)-mimicking DNAzyme sequence. The sequences **1** and **2** form a stable duplex by allosteric, synergetic stabilization of each duplex by the other duplex, while the single-stranded loop can accommodate Pb^{2+}. In the presence of Pb^{2+}, the DNAzyme cleaves the substrate. The separated duplex regions lack thermal stability and the two duplex regions are separated. The release of the cleaved single-stranded products include the HRP-mimicking DNAzyme sequence, and these assemble in the presence of hemin to the catalytic G-quadruplex that catalyzes the oxidation of $ABTS^{2-}$, 2,2′-azino-bis(3-ethylbenzothiazoline)-6-



Scheme 1 Analysis of Pb^{2+} ions or L-histidine by a DNAzyme cascade.

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† Electronic supplementary information (ESI) available: Details on the optimization of the ratio **1** : **2** for highest absorbance signal of the sensing system. See DOI: 10.1039/b716774a

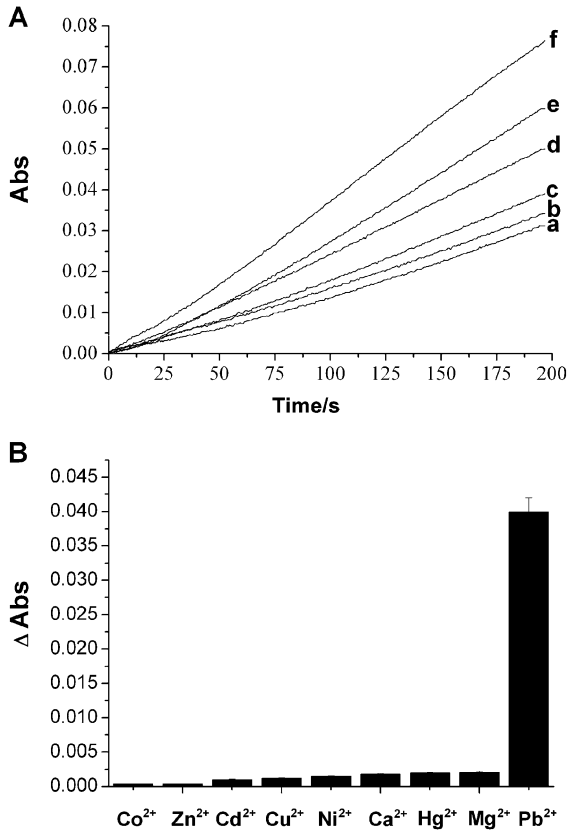

Fig. 1 (A) Time-dependent absorption changes upon the analysis of different concentrations of Pb^{2+} according to Scheme 1: (a) no Pb^{2+}; (b) 1×10^{-8} M; (c) 1×10^{-7} M; (d) 5×10^{-7} M; (e) 1×10^{-6} M; (f) 1×10^{-5} M. In all experiments: [**1**] = 2.78×10^{-7} M; [**2**] = 6.9×10^{-8} M; [hemin] = 1.33×10^{-7} M; [$ABTS^{2-}$] = 2 mM; [H_2O_2] = 2 mM. All experiments were prepared in a Tris-acetate buffer that included 50 mM NaCl, pH = 6.4. (B) Absorbance changes observed upon the activation of the cleavage of **2** by the nucleic acid **1** in the presence of Pb^{2+}, 1×10^{-5} M and a series of other metals, each 1×10^{-5} M. Absorbance changes correspond to the absorbance generated by the system after 2.5 min in the absence of any ion subtracted from the absorbance generated by the system after 2.5 min in the presence of the respective ion.

sulfonate, to the colored product, $ABTS^{\bullet -}$ (λ_{max} = 414 nm). Thus, the analysis of Pb^{2+} can be followed by the colorimetric assay of the released HRP-mimicking DNAzyme units.

Fig. 1(A) depicts the time-dependent absorbance changes in the system upon analyzing different concentrations of Pb^{2+}. The Pb^{2+} can be detected with a detection limit corresponding to 1×10^{-8} M. The analysis of Pb^{2+} by the DNAzyme cascade is selective, and no absorbance changes could be observed upon analyzing other ions at high concentrations (1×10^{-5} M). A series of metal ions (Co^{2+}, Cu^{2+}, Ni^{2+}, Ca^{2+}, Hg^{2+}, Zn^{2+} and Mg^{2+}) yields the background color changes observed in the absence of added Pb^{2+}, Fig. 1(B). The DNAzyme cascade for analyzing Pb^{2+} seems to enhance significantly the sensitivity for analyzing Pb^{2+} ions as compared to other DNAzyme assays.[2–4] It should be noted that the results depicted in Fig. 1 revealed reproducibility within ±5% in a set of five experiments.

The activation of the DNAzyme cascade by Pb^{2+} ions was also followed by the generation of chemiluminescence by the

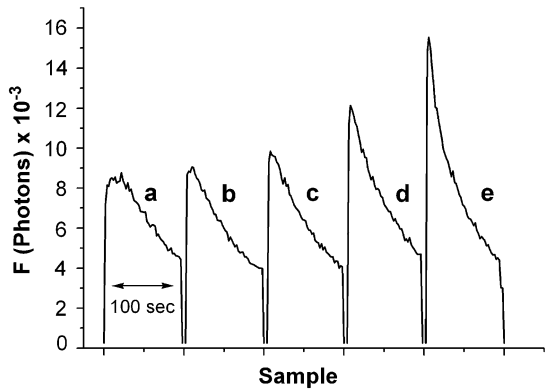

Fig. 2 Time-dependent chemiluminescence generated upon analysing of Pb^{2+} according to Scheme 1: (a) no Pb^{2+}; (b) 1×10^{-8} M; (c) 1×10^{-7} M; (d) 1×10^{-6} M; (e) 1×10^{-5} M. In all experiments: [**1**] = 2.78×10^{-7} M; [**2**] = 6.9×10^{-8} M; [hemin] = 1.33×10^{-7} M; [luminol] = 2 mM; [H_2O_2] = 2 mM. All experiments were prepared in a Tris-acetate buffer that included 50 mM NaCl, pH = 8.2.

horseradish peroxidase-mimicking DNAzyme. In these experiments, released hemin-containing DNAzyme catalyses the oxidation of luminol by H_2O_2, and the generation of chemiluminescence, Scheme 1. Fig. 2 depicts the light intensities generated upon analyzing different concentrations of Pb^{2+} by the DNAzyme cascade system. The association of Pb^{2+} to the **1**/**2** complex stimulates the cleavage of **2** and the release of the HRP-mimicking DNAzyme sequence that folds to the G-quadruplex/hemin biocatalytic structure that catalyses the chemiluminescence reaction. As the concentration of Pb^{2+} is lower, the intensity of the chemiluminescence decreases. It should be noted that the conditions for analyzing Pb^{2+} by the complex **1**/**2** represent optimized conditions (see ESI†) in that we use a ratio of **1** : **2** that corresponds to 4 : 1 in order to preserve the horseradish peroxidase-mimicking DNAzyme units in a blocked inactive configuration.

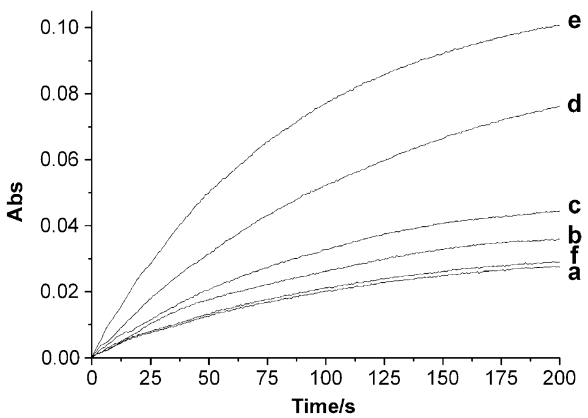

Fig. 3 Time-dependent absorption changes upon the analysis of different concentrations of L-histidine according to Scheme 1: (a) no L-histidine; (b) 5×10^{-4} M; (c) 1×10^{-3} M; (d) 50×10^{-3} M; (e) 0.1 M; (f) D-histidine = 50 mM. In all experiments: [**3**] = 2.78×10^{-8} M; [**2**] = 6.9×10^{-8} M; [hemin] = 1.33×10^{-7} M; [$ABTS^{2-}$] = 2 mM; [H_2O_2] = 2 mM. All experiments were prepared in a HEPES buffer that included 500 mM NaCl, pH = 7.5.

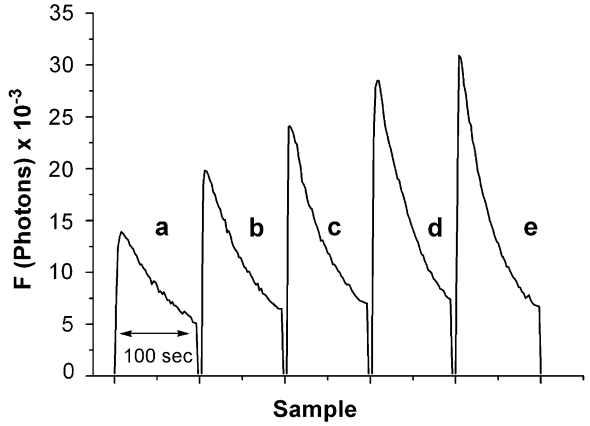

Fig. 4 Time-dependent chemiluminescence generated upon the analysis of different concentrations of L-histidine according to Scheme 1: (a) no L-histidine; (b) 1×10^{-3} M; (c) 20×10^{-3} M; (d) 50×10^{-3} M; (e) 78×10^{-3} M. In all experiments: [**3**] = 2.78×10^{-8} M; [**2**] = 6.9×10^{-8} M; [hemin] = 1.33×10^{-7} M; [luminol] = 2 mM; [H_2O_2] = 2 mM. All experiments were prepared in a HEPES buffer that included 500 mM NaCl, pH = 9.

The method was further applied to detected L-histidine using the L-histidine cofactor-dependent nucleic acid cleaving DNAzyme.[10] The nucleic acid **3** includes the L-histidine recognition sequence, and its 3′ and 5′ ends are tethered to nucleic acids complementary to segments of the HRP-mimicking DNAzyme. The hybridization of **3** with its nucleic acid substrate **2** yields the duplex regions and the loop structure that binds the L-histidine cofactor. Upon binding of L-histidine, the DNAzyme is activated, and the cleavage of the substrate **2** results in the dissociation of the HRP–DNAzyme sequences that self-assemble in the presence of hemin to the biocatalyst that mediates the oxidation of $ABTS^{2-}$ by H_2O_2, or catalyses the generation of chemiluminescence in the presence of H_2O_2/luminol. Fig. 3 shows the time-dependent absorbance changes resulting upon analyzing different concentrations of L-histidine by means of the duplex **3**/**2**, and the activation of the enzyme cascade.

Interestingly, the activation of the enzyme cascade is chiroselective, and upon the interaction of the duplex **3**/**2** with D-histidine, only the background absorbance could be detected, Fig. 3, curve (f).

The activation of the cascades of the DNAzymes by L-histidine was also followed by the generation of chemiluminescence, Fig. 4. As the concentration of L-histidine is lower, the intensity of chemiluminescence decreases.

To conclude, the present study has introduced a new paradigm for analyzing cofactor units of DNAzymes by the activation of a cascade of DNAzymes. The cascade provides an amplification route for the rapid detection of the respective DNAzyme cofactors. It should be noted that the substrate **2** is a versatile nucleic acid sequence that may be conjugated to other metal or amino acid-dependent DNAzymes.

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