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A highly sensitive and selective electrochemical aptasensor for mercury(II) based on the formation of unique ternary structure of aptamer-Hg²⁺-neutral red

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A highly sensitive and selective electrochemical aptasensor for mercury (II) has been developed based on the formation of a ternary complex between mercury (II) specific aptamer (MSA), neutral red (NR) molecule and Hg²⁺ ion without pre-modification of the probe aptamer.

Functional nucleic acids acted as ligand-binding agents (aptamers) attract increasing attention in biosensor construction, because they can specifically recognize various targets through tuning the base sequences. To be specific, the thymine-thymine (T-T) mismatches in DNA duplexes can selectively and tightly bind with Hg²⁺, to form thymine-Hg²⁺-thymine (T-Hg²⁺-T) mediated DNA duplexes. Based on this feature, the T-rich oligonucleotide has been frequently applied as mercury(II) specific aptamer (MSA) for electrochemical sensing analysis of Hg²⁺. For example, Liu et al. have exploited an electrochemical Hg²⁺ sensor using ferrocene (Fc)-tagged MSA as the sensing element. When the MSA was interacted with Hg²⁺ and underwent conformational switch from flexible single-stranded state to the rigid duplex through T-Hg²⁺-T coordination chemistry, the electroactive Fc tag was drawn away from the electrode surface, resulting in the decrease of the redox peaks of Fc tag. Wu et al. have also developed a Hg²⁺ electrochemical sensor based on MSA and its Fc-tagged complementary strand from the electrode surface. As a result, the redox peak currents of Fc tags substantially decreased. More recently, Xuan et al. have developed a immobilization-free and Exonuclease III-assisted amplified strategy for Hg²⁺ detection. In that assay, a methylene blue (MB) modified MSA was used as the electroactive capture probe for Hg²⁺, and then the electrochemical response of MB that released from Exonuclease III digested T-Hg²⁺-T mediated DNA duplexes was utilized for Hg²⁺ monitoring.

However, all these sensors have one common characteristic, i.e., the involved oligonucleotide including MSA or its auxiliary strands need to be pre-labeled with an electroactive signal molecule (like Fc or MB) for signal output. This on one hand increases the preparation cost and operation time of the sensor since the biojunction process is usually sophisticated and complicated, and on the other hand the analytical sensitivity of the sensor is seriously restricted as one MSA strand can only be labeled with one signal molecule. To overcome these disadvantages, some electroactive intercalator-based aptasensors were constructed. In these sensors, the complicated labeling procedures of the signal molecules are not needed, and meanwhile the higher sensitivity was produced through the “signal-on” mode. But because these sensors just work on the dependence of the relatively weak intercalation of the signal molecules into the target-bound aptamers, the signal stability and reproducibility are usually limited during the operation processes of rinsing and measurements. In this communication, we developed a novel and facile electrochemical Hg²⁺ aptasensor based on the formation of a stable ternary complex between MSA, neutral red (NR) and Hg²⁺. Scheme 1 shows the construction strategy of the biosensor and the detailed working mechanism and fabrication procedures were provided in the Electronic Supplementary Materials (ESI).

Scheme 1 Schematic representation of the preparation and working principle of the Hg²⁺ aptasensor based on the aptamer-Hg²⁺-neutral red ternary

Fig.1A shows the typical voltammetric characteristics of the aptasensor in response to Hg²⁺. From the figure, it was clearly observed that not any Faradic current response was observed at Hg²⁺/MCH-MSA/AuE (curve a), suggesting that the film of Hg²⁺/MCH-MSA is electro-inactive under the measured conditions. However when Hg²⁺/MCH-MSA/AuE was measured after incubating in NR solution, a pair of well-defined redox peaks was observed at -0.56 V and -0.62 V (curve c), respectively, which was in good accordance with the characteristic electrochemical response of NR. This result demonstrated that the electrochemical response of the sensor is strictly depended on the presence of Hg²⁺.

The scan rate (v) experiments further showed that the redox
binding amount of NR on the sensor was calculated through the peaks of NR enhanced regularly with the increase of the scan rate. Planar aromatic molecule of NR could intercalatively interacted with duplex DNA within the system of MSASHg2+. However, when the duplex DNA was interacted with intercalators, the DNA strands would be lengthened to accommodate the intercalated molecules, and as a result the absorption intensity of the DNA would be enhanced. Therefore the hyperchromic effect observed for MSA-Hg2+ upon interaction with NR testified that NR bound to MSA-Hg2+ through the typical intercalative mode.

Furthermore, the influence of the MSA-Hg2+ system on the visible absorption of NR was investigated. It could be observed that NR had an intense absorption peak at 538 nm (curve a, Fig. 2B), which was consistent with the absorption wavelength of NR at previous reports. When MSA was added into the NR solution, only a slight decrease of the peak intensity was observed (Fig. 2B, curve b), likely due to the weak electrostatic interaction of the cationic NR with the phosphate backbone of MSA. It was interesting that if the mixture solution of MSA-Hg2+ system was added, a significant blue-shift effect accompanied by the hypochromicity was observed (Fig. 2B, curve c), which was absolutely opposite to the case observed for NR intercalating into natural dsDNA. Therefore, there should be another binding mode existing between NR and MSA-Hg2+ besides the above-mentioned intercalation. It had been reported that the coordination action between Hg2+ and the functional group of chromophore could induce the decrease of n-electron density of the chromophore. As a result, the blue-shift of the characteristic peak of the chromophore could be observed. So, combing the above UV and visible spectra experiments, the binding mode of NR to MSA-Hg2+ could be supposed as the co-existence of intercalation and coordination action.

![Figure 2](image-url)

Fig. 2 (A) UV spectra of 0.1 µM MSA (a), 5.0 µM NR (b), mixture of 0.1 µM MSA and 5.0 µM NR (c) before and after interaction with 5.0 µM Hg2+ (d). (B) Visible absorption spectra of 5.0 µM NR (a), mixture of 5.0 µM Hg2+ and 0.1 µM MSA (b), and the mixture of 5.0 µM NR, 0.1 µM MSA and 10 µM Hg2+ (c).

Under the optimal conditions (see ESI), the DPV response of the developed aptasensor to various concentrations of Hg2+ was depicted in Fig. 3A. It was observed that when Hg2+ was absent, no obvious Faradic current signal was detected, suggesting a negligible background interference of the sensor. With the increase of Hg2+ concentrations (C_{Hg^{2+}}), the DPV response corresponding to NR increased accordingly, suggesting that increasing amounts of NR molecules were accumulated within the sensing layer by the formed Hg2+-MSA duplex structure. The oxidation peak currents (I_{pox}) of NR presented a linear relationship with the logarithm value of C_{Hg^{2+}} (Log C_{Hg^{2+}}) over the range from 2.7×10^{-11} M to 8.5×10^{-9} M. The linear regression equation was $I_{pox} = -1.8543 \log C_{Hg^{2+}} - 22.5215$ with a correlation coefficient ($r$) of -0.9852 (Fig. 3B). Based on 3σ (σ represents the standard deviation of the blank samples, n=7), the...
limit of detection was estimated to be $1.5 \times 10^{-12} \text{M}$ (amounting to 0.3 ppt), which was obviously lower than that of the other MSA-based electrochemical approaches (see Table S1 in ESI). Such a low detection limit was also much lower than the toxicity level of $\text{Hg}^{2+}$ defined by the US Environmental Protection Agency (EPA) in drinkable water ($<10 \text{nM}$, amounting to 2 ppb), suggesting that the developed approach had great promising for the sensitive monitoring of $\text{Hg}^{2+}$ in water sample.

In conclusion, we have developed an efficient and label-free electrochemical sensor for $\text{Hg}^{2+}$ based on the transformation of MSA from random coil to the hairpin-like structure by $\text{Hg}^{2+}$ and the formation of unique electroactive ternary complex of MSA-$\text{Hg}^{2+}$-NR. Because both the binding of MSA to $\text{Hg}^{2+}$ and the formation of the ternary complex are specific, the sensor showed excellent selectivity and ultralow background response during testing. Compared with the conventional labeling technology-based aptasensor, the label-free strategy developed in this communication was simpler and more labor saving. Moreover, since one $\text{Hg}^{2+}$ bond MSA strand could associate with eight probe molecules of NR, the aptasensor could detect the target $\text{Hg}^{2+}$ as low as 1.5 pM, which suggested that the sensor poses great promising in practical environmental monitoring.

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Notes and references

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