ISSN 2959-409X

Electrochemical Biosensor for Detecting both Pb^{2+} and Hg^{2+} in Human Blood

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Abstract:

Many studies have demonstrated that lead ion (Pb^{2+}) and mercury ion (Hg^{2+}) are harmful to human health. Lead was found to accumulate in the kidney, leading to oxidative stress, apoptosis, and inflammation in the nephrons, and possess marked testicular toxicity. Mercury is well-known to produce nephrotoxicity, inflammatory responses and induce oxidative stress and apoptosis. These heavy metals can enter the human body in various ways, such as through skin or inhalation routes, or contaminated drinking water and food. But heavy metal poisoning is not easy to diagnose because existing detecting measures has many disadvantages cause them can't widely spread. So, seeking an easy way to detect them in human blood to discover lead or mercury poisoning is an unmet need. This paper develops a new reusable biosensor for detecting both Pb^{2+} and Hg^{2+} in human blood. This work is based on the reaction between guanine and Pb^{2+} form of G-quadruplex and Hg^{2+} connect two thymines together cause the variation of electrochemical signal. The signal is magnified by methylene blue-gold nanoparticles (MB-AuNP) to increase the limit of detection Although the reuse part of sensor is still in theory, but this approach can make the cost of sensor low and create the feasibility of recycle. Overall, providing a new idea to make contributions to the prevention of heavy metal poisoning is the reason why this study is conducted.

Keywords: electrochemical biosensor, lead, mercury.

1. Introduction

Lead and mercury belong to heavy metals. The accumulation of heavy metals in water becomes a concern. Heavy metals are a kind of biodegradable macromolecules that can afect the activities of proteins and enzymes in the human body [1-3]. Four common approaches detecting heavy metals are:

1. Atomic absorption spectrometry (AAS) is based on the transition of atomic level to measure the content of heavy metals in sample contain when wavelength of the sample will absorb is detected [4].

2. Atomic Fluorescence Spectroscopy (AFS) is a way which has high sensitivity. It is used to measure heavy metals sample contains by atomic fluorescence emission. Atoms will give off a specific wavelength of fluorescence when an appropriate wavelength of light excite it. By detecting the intensity of a sample's fluorescence at a particular wavelength, this method can determine the amount of heavy metal what sample contain [4].

3. Inductively coupled plasma emission spectrometry (ICP – AES): When sample back to ground state after exciting to high energy state by high frequency electric current, it will give off a specific wavelength of light. The wavelength of light can be detected to find how much heavy metal does the sample contain [4].

4. Inductively coupled plasma mass spectrometry (ICP – MS): Sample is transferred to ions by electric field at high temperature. After transporting them to mass spectrometry, magnetic field will separate them because each heavy metal ion possesses its unique mass-charge ratio.[4]

In conclusion, these four approaches need expensive equipment and complicated steps to operate. These disadvantages because they can't be widely spread. Seeking a cheap, fast, and accurate approach to detecting heavy metal is an unmet need. The electrochemical approach is a good choice, because the signal from the sensor can be received by an electrochemical workstation, which is not expensive, and because it is easy to analyse the signal. Biological elements, like the application of DNA, RNA and protein can make the cost low, because they are easy to purchase and prepare. Hence, choosing an electrochemical biosensor to detect heavy metals seems to be a better way to research and study.

The new sensor is based on two core principles. The prin-

ciple of detecting Pb^{2+} is that Pb^{2+} ion chelates with single strand G-rich aptamer to form G-quadruplex (see figure 1) [5]. On the other hand, principle of detecting Hg^{2+} is from specific coordination interaction between Hg^{2+} and double thymine, and advanced Hg^{2+} mismatches with T-T to form stable T-Hg^{2+}-T base pairs (see figure 2) [6,7]. An electrochemical biosensor detecting both Pb^{2+} and Hg^{2+} [3] provides the inspiration of combination of two DNA strands together (one strand for detecting Pb^{2+} and the other for Hg^{2+}). The "sandwich" structure between electrode-DNA strand-signal amplifier comes from an electrochemical biosensor detecting Hg^{2+} [8]. About the signal amplifier, methylene blue - gold nanoparticles (MB-AuNPs) from an electrochemical biosensor detecting Pb^{2+} was adapted [9].

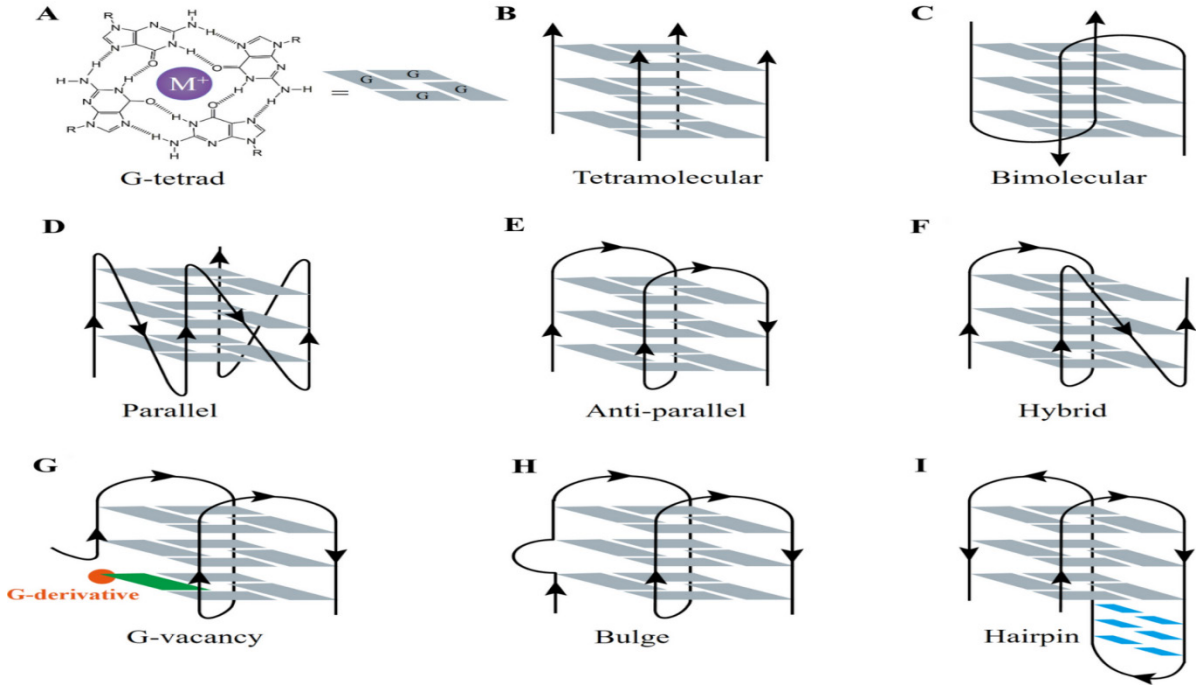

Figure 1. Structures and topologies of G-quadruplex DNA.

A: The distribution of metal ion and guanines in G-quadruplex. Four guanines siege a central cation and they form hydrogen bond to each other.
B, C: Intermolecular G-quadruplex structures: representative topologies.
D–F: The conventional intramolecular G-quadruplex configurations are shown schematically. These G4 structures can be classified as parallel, antiparallel, or hybrid based on the orientation of the G4 sequences.
G–I: Non-canonical intramolecular G-quadruplex structures shown schematically [7].

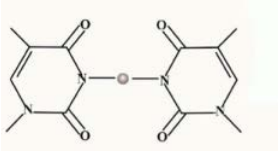

Figure 2. T-Hg^{2+}-T structure [5].

2.Components of sensor

2.1 MB-AuNPs (methylene blue-gold nanoparticles)

Methylene blue (MB), a substance which possesses high electrochemically activity in redox reactions, because of the electron-rich sulphur and nitrogen heteroatoms in its structure, has been widely used in the creation of electrochemical biosensors (Duan et al., 2021). With their exceptional electrical conductivity, exceptional biocompatibility, huge specific surface area, and improved biomolecule absorption, gold nanoparticles have the potential to significantly improve methylene blue stabilisation.
[9].
According to these advantages, MB – AuNPs is adopted to this electrochemical biosensor to enhancing electrical signal from aptamer.
MB – AuNPs is made from mixing DTAB (Dodecyltrimethylammonium bromide) (5mL, 1.14nM), hydrochloric acid ($500\mu L$, 0.1nM) and MB (1mL, 9.8nM) together

to form micelles, then mixing with $HAuCl_4$ (chloroauric acid) (24 0μL , 100mM) at room temperature for 6 hours. Product is stored in 0.1M phosphate buffer solution (PH=7) at 4¡æ .

2.2 DNA strand

The sensor needs 3 kinds of DNA strand, which are S1 , S2 and S3 .

1. S1: 5' - ACTGTTTGTTTGCCCG-CCC - SH - 3'

It contains 3 parts : random code part , T-rich part (both of them are complement of S2) and C-rich part (complementary to S3) . Thiol group at its end can attach with MB- AuNPs.

2.S2: 5' - SH - TTTCTTTCAGT - 3'

Base pair ' CAGT ' is complementary to random code part of S1 , so it can combine with S1 . Its T-rich part will form T- Hg^{2+} -T structure with T-rich part of S1.

3. S3: 5' - GGGCGGGC - 3'

It is a G-rich strand which will form G-quadruplex when Pb^{2+} exist so it can't combine with t he C-rich part of S1.

2.3 Electrode

Gold foil was chosen as electrode due to its good conductivity ($45.2\times10^{6}\Omega m^{-1}$) and the property of Au-S bond formation to let aptamer attach to the electrode.

2.4 Mercaptoethanol

Mercaptoethanol (MCH), which contains both alcohol and thiol groups, is soluble in water and can form Au-S bond. In this sensor, MCH is used to enclose the rest o the active site of electrode and MB-AuNPs, preventing other components in the sample interfering with the electrochemical signal.

3.Installation of sensor

Step 1: Combine MB-AuNPs and S1 by mixing solutions of them together. At this time, Au-S bond will form between gold nanoparticle and thiol the group of S1.

Step 2: Combine S2 and electrode together by soaking electrode in S2-containing sloution . Also, this combination relies on Au-S bond between them.

Step 3: Soak electrode in S1/MB-AuNPs solution (product of S1), let the random code part of S1 and S2 combine. Random code part acts as glue, because two T-rich strands can't combine without Hg^{2+} .

Step 4: Add S3 solution to electrode to combine S3 and a part of S1 (C-rich part).

Step 5: Add MCH solution to electrode to enclose the rest active site of gold electrode and gold nanoparticles.

The final product of sensor is shown in figure 3.

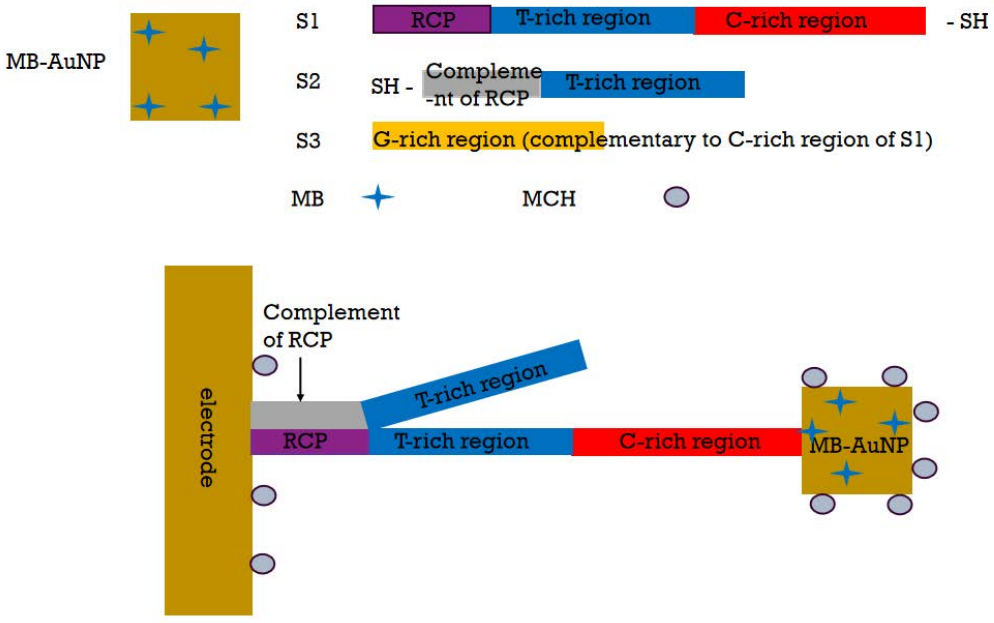

Figure 3: The final product of sensor.

Tips: RCP is the abbreviation of 'random code part'

4.How the sensor work

4.1. Detection of Pb^{2+}

When Pb^{2+} exist in solution, S3 will detach from S1, form G-quadruplex with Pb^{2+} , so the amount of G-rich strand on the electrode decreases, and current signal increased [3]. Figure 4 shows how sensor will change when it meets Pb^{2+} .

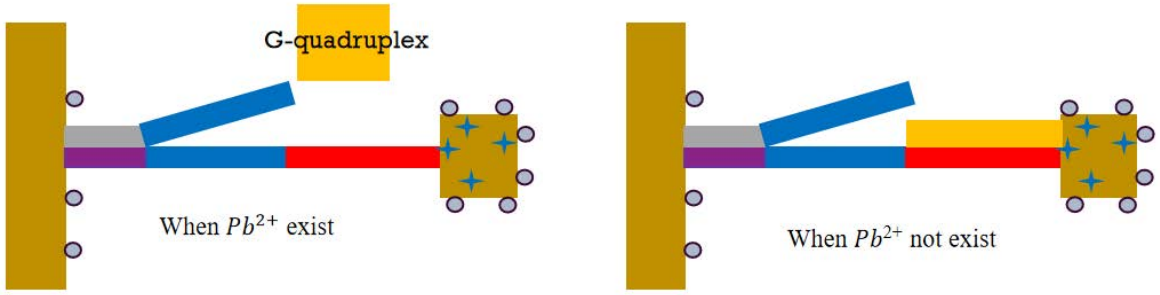

Figure 4. How the sensor changes when react with Pb^{2+}.

4.2. Detection of Hg^{2+}

When Hg^{2+} exist, T-rich parts of S1 and S2 will combine together to form T-Hg^{2+}-T structure . The increase of Hg^{2+} on the electrode obstructed the electron transfer and led to the reduction of current signal [3]. Figure 5 shows the reaction between sensor and T-Hg^{2+}-T .

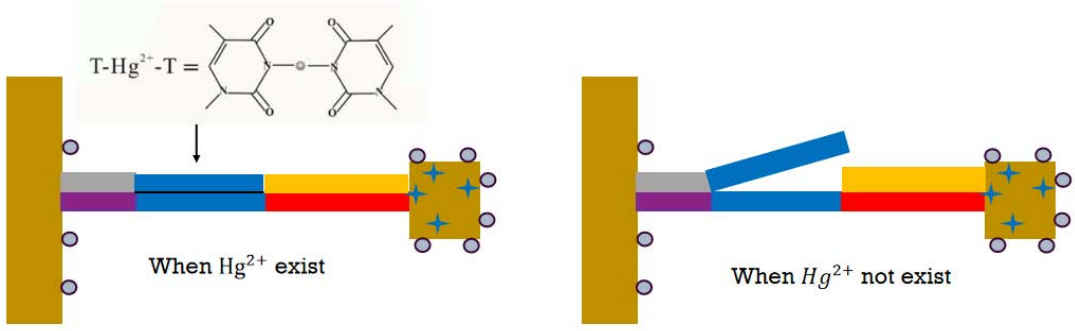

Figure 5: the reaction between sensor and T-Hg^{2+}-T.

4.3 Process of reuse

The process of reuse contains two parts: addition of S3 and breaking T-Hg^{2+}-T structure. It is easy to add excess S3 to react with all Pb^{2+} in solution so a part of S3 will combine with S1 again to reduce the lead-detecting part of S1. In terms of breaking T-Hg^{2+}-T structure, many reagents can make Hg^{2+} insoluble and precipitate, like ammonium solution (NH_4OH), some sulfide and chloride, but which agent is the best needs experimental data.

5.The reason of design

5.1 MB-AuNPs

This sensor is based on electrochemical signal, so to generate a detectable signal, a signal amplifier is necessary. The reason why chooses MB-AuNPs, not metal organic frameworks (MOFs) or others is, AuNPs are relatively small particles, although they are still much bigger than DNA strands. But their size can still provide stability for the sensor. DNA strand is not a very strong connection, so it is difficult to attach some massive particles.

5.2 Sandwitch structure

See figure 3, electrode and MB-AuNPs like two pieces of bread, sandwitch the aptamer. Electric potential between two 'bread' will change when reaction take place in connection (aptamer). This structure is stable and easy to establish.

5.3 Random code part

The random code part acts as glue, connecting S1 and S2 together. The reason why this design is adopted is that this structure is easy to establish, just need to mix solutions that contain each strand together. Also, hydrogen bonds can provide enough strength to hold MB-AuNPs.

6.Conclusion

In conclusion, this report mentions a new conception of electrochemical biosensors to detect both Pb^{2+} and Hg^{2+} in human blood. It is based on two core principles: 1.G-quadruplex will form between G-rich strand and Pb^{2+} ; 2. Hg^{2+} and T-rich strand will form T- Hg^{2+} -T structure. When the sensor react with two metal ions, an electrochemical signal peak will become higher because the existence of Pb^{2+} and another peak will become lower because existence of Hg^{2+} .This can provide a new idea for a cheap, accurate and reusable approach to detecting heavy metal ion in human blood. Contribute for prevention of heavy metal poisoning is the intention of design. Although how to remove Hg^{2+} still not resolved (need experiment to find), but this approach may be improved or referenced by others in order to realize the original intention of the design.

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