Application of fluorescent biosensors for heavy metal ions detection

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Abstract. Heavy metal ions can be successfully detected by using a diverse of the developed fluorescent biosensors. A major trend in designing current fluorescent biosensors incorporates nanomaterials, such as quantum dots. Combined techniques with aptamers allow for highly sensitive analyses of different heavy metal ions. Other novel methods of detection involve whole cells, for example, bacterial bioreporters. A common way of exhibiting fluorescence is through "signal-on" and "signal-off", where some fluorescent biosensors may depend on only one state, others rely on both states of emission. The methods detecting mercury ions listed include mercury-specific oligonucleotide (MSO) probes and a dual emission ratiometric fluorescent probe using silicon (SiNPs) or gold nanoclusters (AuNCs). Methods mentioned for arsenic ions include two different kinds of fluorescent-based aptasensors and a fluorescent DNA quantum dot. As to lead ions, a fluorescent method that utilizes the interactions between DNA aptamer, acridine orange, and nanomaterials is designed. Another method is through quantum dots and DNAzyme synergetic catalytic amplification. For cadmium ions, a whole-cell bioreporter was developed that incorporated bacterial organisms. All recently published methods shared a commonality of exceptional sensitivity and selectivity.

Keywords: heavy metal ions, fluorescent biosensors, detection, sensitivity

1. Introduction

The history of fluorescence observed as a luminescence phenomenon can be dated back to the 17th and 18th centuries. Fluorescence biosensors has gradually developed into a mature sensing technique in the past century. In modern medical testing, biotechnology, and drug discovery, fluorescence biosensing has remained the most popular technique for analytical research. Its diverse implications have allowed the detection of a myriad of biomolecules and bioactivities. Although many types of fluorescent biosensors exist, their core mechanisms remain constant. Excitation light is emitted to the fluorophore or fluorochrome, which evokes the emission of a shorter wavelength, a phenomenon called Stokes Shift. Fluorophores or fluorochromes are molecules that hold a spectrum of absorption and emission characteristics. They have specificity toward binding targets, namely proteins, lipids, and ions [1].

Fluorescent biosensors have a wide range of implications, and the analyse of heavy metal ions is one. Heavy metal ions are non-biodegradable substances that can cause particular harm to the human body and environment through accumulation [2]. Heavy metal ions can be converted into metal toxins within the body or the food chain, hindering metabolic processes [3]. Low concentrations of heavy metal ions are enough to cause substantial harm. Previous techniques, including atomic absorption spectrometry (AAS) and inductively, coupled plasma mass spectroscopy, have provided analysis of heavy metal ions. Yet these methods hold limitations for stability, selectivity, and detection limits with aqueous solutions. Attention has been turned towards optical detection methods, especially fluorescence biosensors, with their prominent features in simplicity, selectivity, and low-detection limits [2].

An always existent challenge with the development of fluorophores occurs in the development of fluorescent probes. Factors that need to be considered during the development of probes are their affinity and compatibility with aqueous solutions. There is great uncertainty in the ability of the probe to succeed in its purpose even if appropriate structures are designed and synthesized [4]. Another problem in fluorescent biosensing is the limitation in resolution. Fluorescent microscopy cannot

penetrate below the molecule's diffraction limit. Diffraction limits tend to be what is halved of the emitted light, meaning that fluorescence microscopy is restricted by the wavelength of the emitted light. The interactions between fluorochromes at proximal distances are present and unsolved. Additionally, various fluorochromes or probes could potentially reduce the signal-to-noise ratio (SNR) [1]. Progression of fluorescence biosensors towards becoming label-free by directly using natural fluorescence properties has been seen as one of the potential solutions to the mentioned limitations and more [5].

This research aims to summarize fluorescent biosensors in the fields of heavy metal ions detection and analysis. The heavy metal ions included are mercury, arsenic, lead, and cadmium. Conventional methodologies incorporating fluorescence, for instance, quantum dots, will be outlined in terms of their mechanism and relatedness to heavy metal ions. Specific examples concerning each type will be mentioned, yet the will focus on generalizations. Finally, this research seeks to provide the broad direction of future developments in heavy metal ions by using fluorescent biosensors.

2. Applications of Fluorescent Biosensors

2.1. Mercury detection

Mercury (Hg) pollution is one of the most heavily discussed topics in environmental sciences. It is a highly toxic element that can enter marine food chains by converting inorganic mercury into methyl mercury by microorganisms. This causes bioaccumulation of methyl mercury through the food chain. Its high affinity causes mercury's effects on humans to thiol groups. Low concentrations of mercury are enough to cause motor and cognitive disorders [6]. Prior methods of Hg^{2+} detection include atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray absorption spectrometry. New developments in biosensing techniques seek to overcome the limitations existing in these previous methods, such as high time consumption, costly instruments, complicated operational procedures, inability to be transported, and more.

One way of detecting Hg^{2+} is through mercury-specific oligonucleotide (MSO) probes, also known as mercury aptamers. Aptamer and entire cells have evolved to become important biofunctional nanomaterials in bioreceptor designing. The use of hybridized nanomaterials can accomplish an integrated detection system. Oligonucleotides are short DNA or RNA molecules that are chemically synthesized in laboratories. They are inexpensive, practical, ecofriendly, and specific when it comes to the detection of heavy metal ions. Exploiting the specificity of Hg^{2+} to thymine, the T-Hg^{2+}-T oligonucleotide was first created in 2004 by Ono and Togashi using the fluorescent resonance energy transfer (FRET) phenomenon. A quencher and fluorophore were put on the 5' and 3' termini. When in the absence of Hg^{2+}, the MSO had an unorganized conformation, and the distance between the termini was far enough to allow the fluorophore to fluoresce. When added Hg^{2+}, the MSO undergoes a conformational change and forms a hairpin structure, and the distance between the termini enables the quencher to reduce the fluorophore emissions [6].

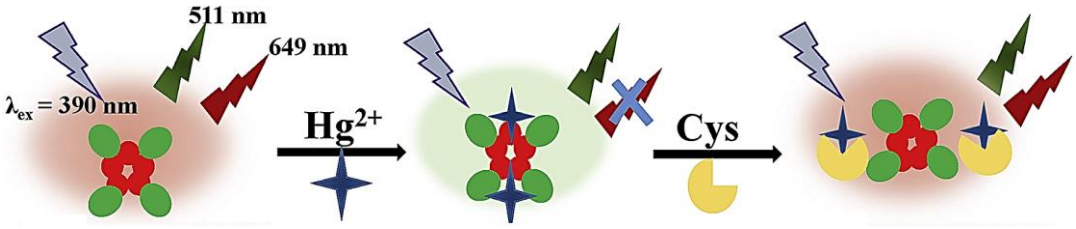

Figure 1 The principle of the developed fluorescent biosensor [8].

Further developments in MSOs have been the incorporation of nanomaterials. Nanomaterials have been selected as quenchers and fluorophores. Quantum dots (QDs) and fluorescent metal nanoclusters (NCs) have been chosen as fluorescent materials.

"Signal-on" and "signal off" detection mechanisms are commonly utilized in MSOs. A dual-emission ratiometric fluorescent probe using silicon (SiNPs) or gold nanoclusters (AuNCs) has been

proposed [8], as shown in Figure 1. Unlike other conventional methods of "turn-off" sensing signals, an "on-off-on" sensing signal was achieved. They chemically connected the red emissive AuNCs to the green emissive SiNPs to allow for dual emission and compared the hybrid to its individual parts. The probe turned out to be highly stable under extreme pH environments and greatly resistant to photobleaching. The "off" sensing signal is displayed as Hg^{2+} has the effect of quenching the red emission while unaffecting the green emission. Under different concentrations of Hg^{2+}, noticeable color changes were observed even when changes were minor, excelling in sensitivity. As to the "on" sensing signal, Cysteine enhances the probe's emission. The probe is exceedingly selective as most other ions or amino acids displayed no signs of quenching or enhancing.

2.2. Arsenic detection

Arsenic (As) is a metalloid or heavy metal that can cause much harm to the environment and the human body. The most commonly observed source of inorganic arsenic is contaminated water. Drinking water contaminated with arsenic has become a great global health concern. Arsenic is harmful to a myriad of organs, including eyes, kidneys, liver, skin, etc., and is likely to induce cancer. Developments in biosensing techniques have been crucial to alleviate and combat the harms caused by arsenic pollution.

Nanoparticles hold many advantages in detecting arsenic. Similar to the previously mentioned nanomaterials-based detection of mercury, nanoparticles are also utilized in a similar way to emit, or quench, fluorescence in the presence of arsenic. Liu et al. reported a new arsenic aptasensor [9]. Arsenate (AsO^{5+}) was detected through DNA absorption by magnetic beads. When AsO^{5+} is absent, the magnetic beads absorb the fluorescently labelled oligonucleotides through the phosphate backbone, which will lead to quenching. In the presence of AsO^{5+}, its high affinity for the fluorophore drew it away from the quencher and thus released fluorescence.

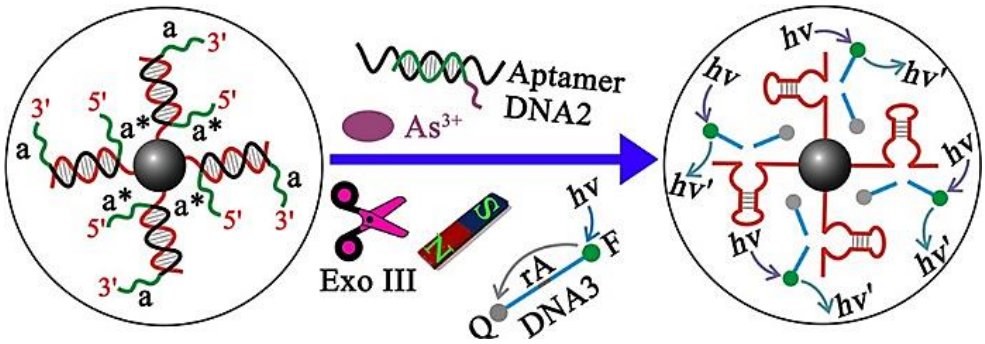

Figure 2 The principle of the developed highly sensitive aptasensor [10].

A more recent arsenic fluorescence-based biosensing method was developed [10], as shown in Figure 2. In the presence of arsenic (III), the complementary strand of DNA is released and obtains a hairpin structure on the streptavidin conjugate. Under this form, strong fluorescence can be observed. In contrast, without arsenic (III), the aptamer retains the complementary strand of DNA and releases significantly lower fluorescence. Advantages to this sensing method include simplicity, quick feedback, and label-free aptamer. Another arsenic fluorescence-based aptasensor uses DNAzyme as a signal amplifier. This biosensing system was designed by Zeng et al. and was targeted toward detecting trace amounts of arsenic (III) [11]. First, a recognition between arsenic (III) and the aptamer sequence is performed. This will cause Exonuclease III (Exo III)-mediated DNA recycling digest process to occur and produce vast amounts of magnesium (Mg^{2+}) dependent DNAzyme in the sensing system. Finally, magnetic separation happens, and active DNAzyme with many turnovers will catalyze the separation of the fluorophore and the quencher substrate strand. After these amplification stages, the fluorescence seen is highly intensified, giving rise to a highly sensitive technique with a detection limit of 2 pM.

Nanomaterials-based fluorescent biosensors have also turned towards quantum dots (QDs) in detecting arsenic. Zhang et al. developed fluorescent DNA quantum dots made under relatively low

reaction temperatures with G-/T-rich ssDNA [12]. The newly formed DNA QDs retained the previous characteristics (basic structure and biological activities) of ssDNA and were able to bind specifically with arsenite (As^{3+}). An organized assembly of the (GT29) region was then created, which caused the stiffening of the assembly structure, hindering the nonradiative relaxation channels and populating the radiative decay. The assembly, under the presence of As^{3+}, became greatly emissive. This technique is a typical "turn on" detection and has the outstanding characteristic of being highly specific.

2.3. Lead detection

Lead is one of the most abundant toxic substances that pose great harm to the environment and human health. Lead's attractive physical qualities have resulted in its wide range of applications in many industries. Up to the late 1980s, organo-lead compounds were still additives to octane and gasoline to increase their combustion. This contributed to the sharp increase in lead pollution [13]. The 300 million tons of lead that have been mined up to now are still circulating throughout the environment, mainly in the soil and groundwater [7].

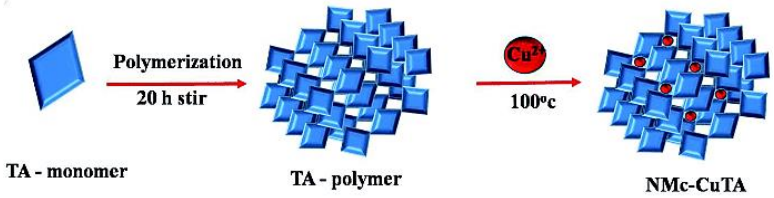

Figure 3 The synthesis of NMc-CuTA [14].

In 1996, Czarnik et al. became one of the first to develop a fluorescent chemosensor for Pb^{2+} [7]. The method included both 2- and 9-anthracene derivatives carrying the N-methylthiohydroxamate ligand (1 and 2), which allowed strong quenching through photo-induced electron transfer (PET). The complexation of 9-derivative 2 with Pb^{2+} gave rise to a 13-fold fluorescent boost at a pH of 9. Although the method was limited in specificity, its impact on future designs has been phenomenal.

Arunjegan et al. created a new fluorescent biosensing method for Pb^{2+} detection with the interactions between aptamer (LFGr-ssDNA), acridine orange (AO), and nanomaterials [14], as shown in Figure 3. Through observation, LFGr-ssDNA and AO exhibit strong adhesiveness to NMc-CuTA through π-π stacking and electrostatic interactions, which causes the fluorescence quenching of AO. When Pb^{2+} is introduced, LFGr-ssDNA specifically binds to Pb^{2+} and forms a G4 complex (G-Pb^{2+}-G base pair) and detaches from the surface of NMx-CuTA with AO fluorescent enhancement (turn-on). The continuation of the detection involves the sensing of epirubicin cancer drugs, which will not be discussed. The detection limit of Pb^{2+} under this method is 1.5 nM, an exceedingly high sensitivity. Other advantages of this biosensor are simplicity, high selectiveness, and being label-free. Another method accounts for the detection of Pb^{2+} through gold-doped carbon dots (CDAu) and DNAzyme synergetic catalytic amplification [15]. Using this method, the limit of detection for target is 0.25 nmol/ L, showing the high sensitivity for detection of Pb^{2+}.

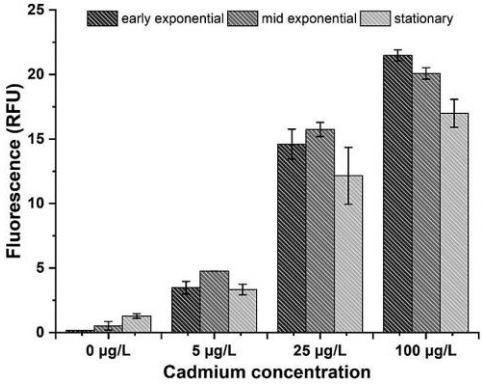

Figure 4 The fluorescence under different cadmium concentrations [16].

2.4. Cadmium detection

Cadmium (Cd) is a heavy metal that can deal harm in trace amounts. Cadmium can be found naturally in the Earth's crust at 0.1-0.5 ppm concentrations. The widespread of Cadmium has been caused by industrial and agricultural processes, such as phosphate fertilizers or batteries, especially in recent times. The resulting consequences of Cadmium spreading have been the contamination of potable water and food sources (meats, grains, etc.). This results in the bioaccumulation of cadmium in food chains, which has significantly been problematic [16].

Further elaborating on its harmful effects, trace exposure to cadmium can cause damage and stress to essential organs, for instance, the brain, kidney, liver, or spleen. Those who ingest cadmium are also more susceptible to acquiring lung cancer. Thus, it is critical to monitor levels of cadmium in the environment [15]. In 2020, Evrim et al. proposed a fluorescent whole-cell bacterial bioreporter [16]. The bacteria Escherichia coli MG1655 (pBR-PzntA), under the control of the zntA gene promoter of heavy metal resistance determinant, expressed green fluorescent protein. Detection for cadmium ion concentrations of 5μg/L was achieved after 3.5 hours, and this number improved to 2 μg/L after another 1.5 hours. The bioreporter is greatly sensitive to cadmium ions and also specific, as shown in Figure 4. The bioreporter is only sensitive to cadmium ions at standard drinking water concentrations. Noted with care was that the bioreporter performed maximum fluorescence for early exponential growth phase cells.

In the same year, Guo et al. developed a mammalian cell-based fluorescent biosensor that gives insight into the synergetic toxicity of cadmium and deoxynivalenol [17]. Previous surveys conducted by Guo et al. reflected that both elements appeared together in many products, and AP-1 partook in the interaction between DON and CD. Taking upon these observations, AP-1 site-mCherry-based bioreporters were constructed. The team tested different combinations of the targets and observed fluorescence signals in the biosensors. In contrast to either individual toxin, together, DON and Cd^{2+} emitted enhanced fluorescence. Listed advantages of this design include 1) Simple observation of multiple toxins at environmental concentrations; 2) Capacity to be a broad-spectrum tool for combined toxicity of DON+Cd; 3) The method generates no pollution and has a stable fluorescence response.

In 2021, Jia et al. proposed another whole-cell biosensor (WCB). Their research also provided a circuit amplification method to enhance sensitivity [18]. After numerous trials, WCB KT-5-R with Pseudomonas putida KT2440 as the host was seen to outperform other candidates. To achieve higher standards of sensitivity, a positive feedback amplifier was added. A detection limit of 0.01 μM was reached when the WCB was equipped with the T7RNAP amplification module, p2T7RNAmut-68. Fluorescent responses were released by the fluorescent reporter, mCherry, integrated into the WCB. Under such a combination, better cadmium tolerance was achieved, and the detection limit appealed to the WHO standard.

3. Conclusion

The development of various fluorescent biosensors has been instrumental in detecting and giving rise to the possibility of alleviating the harms caused by multiple heavy metal ions. Especially in recent times of development in industrial and agricultural works, the widespread of these toxins has brought substantial damage to the environment and its species, humans alike. This research focused on four common heavy metal ions, mercury, arsenic, lead, and cadmium, and provided up-to-date fluorescent-based biosensing methods for sensing these elements. A brief overview of each element and its harms were also given. The biosensors mentioned fall under aptamers, DNAzyme, QDs, CDs, and whole cells bioreporters. Basic mechanisms concerning each specific biosensor were briefly mentioned, along with some characteristics.

Authors' Contributions

Jacob Ze Jia Xu completed the work design and article writing.

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