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The Application of Molecular Materials in Heavy Metal Detection

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Abstract: As the requirements for high environmental and food quality in modern society have increased, rapid heavy metal detection technology has increasingly been applied in sudden event and integrated management systems. In recent years many molecular materials have been applied to the detection of heavy metals due to their variable properties, greatly improving the range and accuracy of detection. The novel approach of introducing inactive DNA probes allows the detection level to be tuned over several orders of magnitude.
Keywords: heavy metal, DNAzyme, DNA aptamer, immune detection

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1. INTRODUCTION

In terms of environmental pollution, heavy metals refer to Pb(lead), Cd(cadmium), Hg(mercury), As(arsenic) and Cr(chromium) and also include those metals which have a certain toxicity such as Cu(copper), Zn(zinc), Ni(nickel), Co(cobalt), Sn(tin), etc. In nature, heavy metals are not easily transformed into nontoxic substances using chemical or biological methods. As a result, they present persistent threats to human health.

Heavy metal pollution in China is a serious issue. The heavy metal soil contents in communities near mining areas and electrochemical plants are several hundreds or even thousands of times higher than inherent soil levels [1-3]. The agricultural land polluted by heavy metals in China is about twenty-five million square meters and the amount of contaminated grain produced in these areas is up to twelve million tons annually. In addition, the production of grain is decreased by ten million tons per year because of this pollution, meaning that the total economic loss is above twenty billion yuan each year. According to a study by the Ministry of Agriculture in China, 64.8% of the total Chinese territory suffers from heavy metal pollution, 6.8% of which is considered severely polluted [4]. Human disease caused by heavy metal contamination of the food chain increases year by year, which indirectly results in huge economic losses. On the whole, it is therefore of the utmost urgency to establish and perfect systems of heavy metal detection and environmental protection.

In general, heavy metals in solution are not easily stabilized [5]. Metal ions can bind to carboxyl, amino and hydroxyl functional groups which provide electrons for coordination. Small amounts of heavy metals such as zinc, copper, manganese and chromium are present in

the body as metalloenzymes. In plants, proteins that are known to combine with heavy metals include the metallothioneins and the phytochelatins [6]. In bacteria, fungi and other microorganisms, people have extracted chitin and developed derivatives which can bind manganese and lead for use in wastewater treatment and medical research [7-9]. In recent years, an increasing number of small molecules have been used in heavy metal detection and removal. Because of their properties such as high surface area, the absorption of heavy metal is more efficient than by macromolecular materials. They have been widely used in the detection of heavy metal pollution, especially trace detection.

2. NUCLEIC ACID PROBES FOR HEAVY METAL IONS

2.1 Deoxyribonuclease (DNAzyme)

Deoxyribonuclease(DNAzymes) may provide a general solution for metal detection due to relatively high stability, low cost, and easy synthesis. In 1994, Breaker and Joyce obtained a population of single-stranded DNA molecules which catalyzed efficient Pb(II)-dependent cleavage of a target RNA phosphoester after five rounds of in vitro selection, which is the original procedure of Systematic Evolution of Ligands by Exponential Enrichment (SELEX). The DNA molecules found here were DNAzymes. As shown in Fig. 1, the fluorophore labeled at the 5'-end of the substrate strand and the quencher labeled at the 3'-end of the enzyme strand contacted each other, and then fluorescence of the fluorophore was quenched. When Pb(II) was present, cleavage of the substrate strand separated the fluorophore from the quencher, resulting in restoration of the fluorescence. The limit of detection for the DNAzyme-based homogenous Pb(II) sensor was 3.1 nM of metal ions [10].

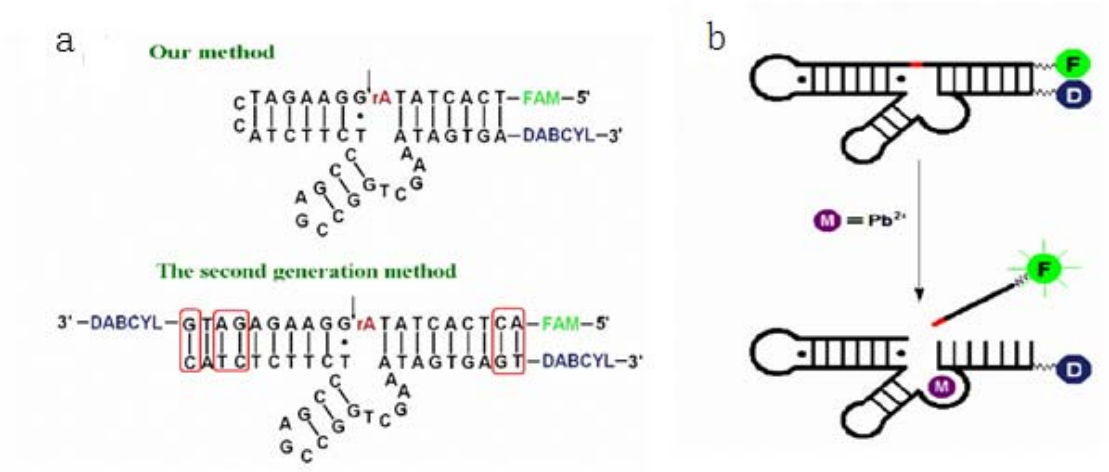

Fig. 1 Design of a single-stranded DNAzyme-based fluorescent sensor for Pb(II) detection (from Li et al., 2012[10])

With regard to the DNAzyme in the colorimetric detection of metal ions, Yin *et al*. reported the development of a versatile allosteric dual-DNAzyme unimolecular probe with a simple and label-free design. As illustrated in Fig. 2, this unimolecular probe comprises both a DNA-cleaving DNAzyme and a HRP(Horseradish Peroxidase)-mimicking DNAzyme [11]. In the absence of Cu(II) ions, three domains act cooperatively in the DNA-cleaving active state and the resulting structure is more stable than the G-quadruplex structure. Conversely, Domain I would be cleaved to release Domain II which intercalates hemin. The H-DNAzyme (G-quadruplex combined with hemin) transduces the sensing through catalytic H_2O_2-mediated oxidation of 3,3,5,5'-tetramethylbenzidine to form a blue product. Many studies have been reported employing DNAzyme probe detection for Pb(II), Hg(II) or Cu(II) [12-14].

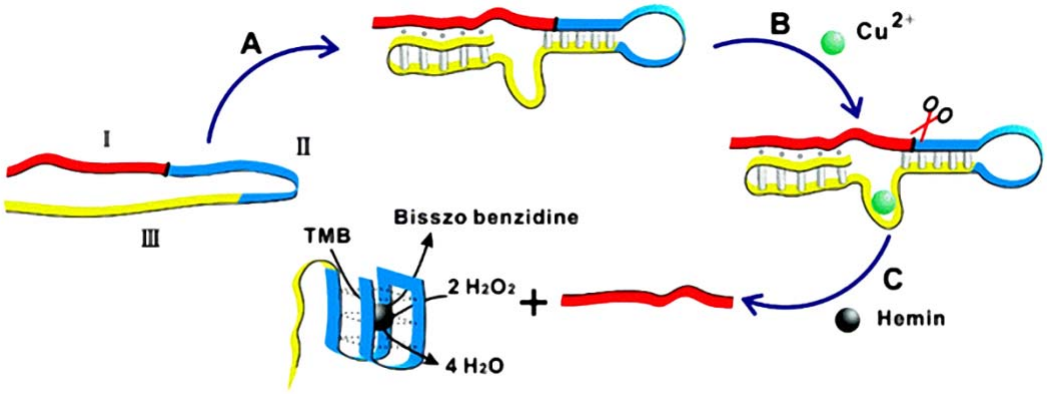

Fig. 2 Working mechanism of colorimetric Cu(II) sensor (from Yin et al., 2009[11]).

2.2 Adapting selected nucleic acid ligands (DNA aptamer)

Adapting selected nucleic acid ligands (DNA aptamers) are oligonucleic acids usually created by exponential enrichment and *in vitro* selection processes. Compared with antibodies, aptamers can be processed more rapidly, exhibit higher affinity and stability and are lower cost. The direct transformation of a molecular signal to an optical signal is the most important property of the DNA aptamer. In the Hg(II)-sensing system, the sensor D-oligodeoxyribonucleotide (ODN)-F, comprises an ODN moiety functionalized with a fluorophore (fluorescein, F) and a quencher (dabcyl, D) and is also rich in thymine-thymine (T-T) base pairs. In the presence of Hg(II) ions, mercury-mediated base pair coupling (i.e., T–Hg–T) are formed between the thymine residues from two Hg-binding sequences in the ODN giving rise to a hairpin structure [15]. Consequently, this results in significant quenching of the fluorescent emission relative to the random coil. The LOD can reach 40 nmol/L (Fig. 3).

Inspired by the recent use of electrogenerated chemiluminescence (ECL) for analyte detection in organic chemistry, the ruthenium(II) complex (Ru1) was developed as an ECL emitting species [16]. Ru1 can approach the electrode surface when the T-Hg-T structure was

formed in the presence of Hg(II), resulting in an increase of the anodic ECL signal. The detection range of this ECL signal is two to three orders of magnitude greater than that of ODN.

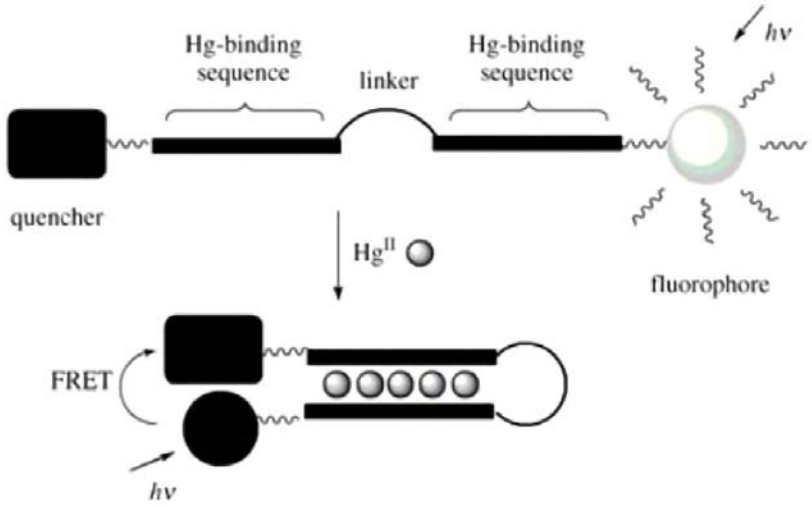

Fig. 3 A schematic representation of the hairpin structure induced in D-ODN-F (dabcyl-oligodeoxyribonucleotide-fluorescein) by Hg(II) mediated T-Hg-T pair formation (from Ono, et al., 2004[15]).

In a study of nanoparticle aggregation, it was found that oligonucleotide-based methods allow the controlled and reversible assembly of gold nanoparticles (AuNPs) into supramolecular structures [17]. Xu *et al*. reported a simple and sensitive colorimetric detection method for Hg(II) ions based on salt-induced aggregation of AuNPs caused by mismatches in the DNA oliognucleotides. This sensing system had a detection range from 0 to 5 μM and an LOD of 0.5 μM [18]. Prior to this, a series of Pb(II)-sensing systems based on the aggregation of nanoparticles were proposed by Liu *et al*. [19] and Zhao *et al*. [9].

Most of the studies discussed were performed in a liquid or soil environment, however some involved investigating metal accumulation in animals. According to the relationship between metal ion concentration and the expression involving microRNA, Suzuki *et al*. detected cadmium through RT-PCR (reverse transcription PCR) for the mRNA expression of metallothionein and estrogen receptors [20]. This discovery indicated that metal ion concentration can also be described by the expression involving DNA or microRNA.

3. ANTIBODIES FOR HEAVY METALS

Immunoassay detection of heavy metals is based on the synthesis of complete antigens that chelate metal ions and the system for collecting signals generated from the specific combination between the antigen and antibody. With a specific monoclonal antibody, immunoassays offer significant advantages over traditional detection methods including high sensitivity, selectivity, rapid, low-cost, easy to carry and species-specificity [21-23]. In 1985,

Reardan *et al*. [24] connected In(II) to keyhole limpet hemocyanin (KLH) *via* a L-benzyl-EDTA linker, which resulted in the creation of the monoclonal antibody against the complex. Since then, monoclonal antibodies selected to preferentially combine with nine kinds of heavy metal ions have been reported.

The chelate provides an organic shell that can be specially recognized by antibodies for the metal ions, as the ions are too small to be effective targets of the immune system. So far, there are five primary chelates (Table 1) for metal ions; there are also some chemical analogues of these six molecules that have been used for metal chelating. The choices of the chelator have an important influence on the association constants between the ions and the chelate, the hapten and the antibody.

Blake *et al*. found that an antibody bound to the metal-chelate-protein conjugate with an affinity approx. 3000-fold greater than that involving the soluble metal-chelate complex, and that the addition of a nitrobenzyl or amino group to the EDTA decreased the K_d by four to five orders of magnitude, relative to the original immunogen. In contrast to original immunogens, the chelate with more complex structure would generate antibodies with higher affinity constants and stronger specific binding abilities [22] .

Jones *et al*. used matrices of different volumes to identify the areas of metal-chelate haptens that differed in three-dimensional space [25]. Khosraviani *et al*. found that antibodies had the ability to interact with different chelates and structurally related derivatives with affinities 50- to 10000-fold less than that determined for the original chelate. These two studies indicated that antibody affinity correlates well with the complexity of chelate structure and that the selection of chelate was the foundation to the immune system [26].

Table 1 Six molecules used for heavy metal chelation

Chelate	Target metal ion
Ethylenediaminetetraacetic acid (EDTA)	Hg(II)
Diethylene triamine pentacetate acid (DTPA)	In(III)
1-(4-Isothiocyanobenzyl)-ethylene-diamine-N,N,N',N'-tetraacetic acid (ITCBE)	Cd(II)
N,N'-1,2-ethanediylbis-1-Aspartic acid (EDDS)	Zn(II)
Nitrilotriacetic acid (NTA)	Cu(II)

A crucial point in the design of heavy-metal-sensing systems is recording the immunoassay signal, which can be done by the immobilization of specific receptors onto an adequate platform [27]. Recently, colloidal gold CIA for metal ion detection have emerged on a large scale. A lateral flow immuosensor device for Cd(II) determination using the Cd-EDTA-BSA-AuNP conjugate to generate the signal was introduced with a LOD of 0.1 pg/L. Tang *et a*l. developed a one-step CIA using a colloidal gold labeled monoclonal antibody

probe for the rapid detection of lead ions in water samples [28]. The results could be easily judged visually based on the presence or absence of a red colored test line. This method showed high sensitivity with a LOD of 50 ng/mL and a limited detection time of 5 minutes.

4. THE APPLICATION OF OTHER MOLECULAR MATERIALS

Other molecular materials have been used in heavy metal detection, such as carbon nanotubes [29], quercetin [30], etc. Because they have high surface areas and are porous. Besides, some water-soluble indenofluorene based oligomers used as new chemosensors for Hg^{2+} were reported [31].

5. DISCUSSION AND CONCLUSION

At present, the detection of heavy metals in soil, water and food still relies on traditional methods such as atomic absorption spectroscopy. However, point-of-care devices are currently being developed to provide low-cost analytical systems useful for environmental monitoring of heavy metals. In this context, paper-based systems are highly novel and important. The advantages of materials combined with paper-based systems have already revolutionized the detection of heavy metals.

In the management of sudden environmental pollution incidents or fast customs processing of goods, biomolecular detection are increasingly finding use. Molecular techniques have been applied to the detection of metal ions with the advantages of simplicity, rapidity, low cost and high sensitivity. In combination with nanotechnology, it is expected to help address problems such as fluid detection in clinical trials and *in-situ* testing. With the continuing development of nanotechnology, there are more nanoparticles being synthesized with good biocompatibility, low toxicity and strong adsorption capabilities. More new methods in combination with small molecular reagents have been established. This will provide more convenient and reliable ways for environmental detection of trace heavy metals, especially for *in-situ* monitoring and real-time detection.

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REFERENCES

[1] H Bai, B Hu, C Wang, et al, “Assessment of Radioactive Materials and Heavy Metals in the Surface Soil around the Bayanwula Prospective Uranium Mining Area in China”, International Journal of Environmental Research and Public Health, 2017, Vol. 14 (3), p300.

[2] N Zheng, J Liu, Q Wang, et al. “Health risk assessment of heavy metal exposure to street dust in the zinc smelting district, Northeast of China”, Science of the Total Environment, 2009, Vol. 408 (4), p726-733.

[3] P Zhuang, B Zou, N Li, et al. “Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, China: implication for human health”, Environmental

[4] B Cui, L Wang, G Zhang, et al. “Review of present situation of the Soil heavy metal pollution and the harm and repair technology”, Journal of Anhui Agricultural Sciences, 2012, Vol. 40 (1), p373-375.

[5] J R Conner. Chemical Fixation and Solidification of Hazardous Wastes, Van Nostrand Reinhold, 1990, Vol. 35, p82-91.

[6] W E Rauser. “Structure and function of metal chelators produced by plants”, Cell biochemistry and biophysics, 1999, Vol. 31 (1), p19-48.

[7] U M Ekanemesang, S Rodriguez-Eaton, J Bender, et al. “Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats”, Applied and environmental microbiology, 1994, Vol. 60 (7), p2311-2315.

[8] R Jayakumar, M Prabaharan, S V Nair, et al. “Novel chitin and chitosan nanofibers in biomedical applications”, Biotechnology advances, 2010, Vol. 28 (1), p142-150.

[9] W Zhao, M A Brook, and Y Li. “Design of Gold Nanoparticle-Based Colorimetric Biosensing Assays”, J. ChemBioChem, 2008, Vol. 9 (15), p2363-2371.

[10] H Li, Q Zhang, D Kong, et al. “Single-stranded DNAzyme-based Pb^{2+} fluorescent sensor that can work well over a wide temperature range”, Biosensors and Bioelectronics, 2012, Vol. 34 (1), p159-164.

[11] B Yin, B Ye, W Tan, et al. “An allosteric dual-DNAzyme unimolecular probe for colorimetric detection of copper (II)”, Journal of the American Chemical Society, 2009, Vol. 131 (41), p14624-14625.

[12] J Elbaz, B Shlyahovsky, I Willner. “A DNAzyme cascade for the amplified detection of Pb^{2+} ions or L-histidine”, Chemical Communications, 2008, Vol.13 (13), p1569-1571.

[13] M Hollenstein, C Hipolito, C Lam, et al. “A highly selective DNAzyme sensor for mercuric ions”, Angewandte Chemie International Edition, 2008, Vol. 47 (23), p4346-4350.

[14] Q Zhang, Y Cai, H Li, et al. “Sensitive dual DNAzymes-based sensors designed by grafting self-blocked G-quadruplex DNAzymes to the substrates of metal ion-triggered DNA/RNA-cleaving DNAzymes”, Biosensors and Bioelectronics, 2012, Vol. 38 (1), p331-336.

[15] A Ono, H Togashi. “Highly Selective Oligonucleotide‐Based Sensor for Mercury (II) in Aqueous Solutions”, Angewandte Chemie International Edition, 2004, Vol. 43 (33), p4300-4302.

[16] F Ma, Y Zhang, H Qi, et al. “Ultrasensitive electrogenerated chemiluminescence biosensor for the determination of mercury ion incorporating G4 PAMAM dendrimer and Hg

(II)-specific oligonucleotide", Biosensors and Bioelectronics, 2012, Vol. 32 (1), p37-42.

[17] C A Mirkin, R L Letsinger, R C Mucic, et al. "A DNA-based method for rationally assembling nanoparticles into macroscopic materials", Nature, 1996, Vol. 382 (6592), p607-609.

[18] X Xu, J Wang, K Jiao, et al. "Colorimetric detection of mercury ion(Hg^{2+}) based on DNA oligonucleotides and unmodified gold nanoparticles sensing system with a tunable detection range", Biosensors and Bioelectronics, 2009, Vol. 24 (10), p3153-3158.

[19] J Liu, Y Lu. "A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles", Journal of the American Chemical Society, 2003, Vol. 125 (22), p6642-6643.

[20] N Suzuki, M Yamamoto, K Watanabe, et al. "Both mercury and cadmium directly influence calcium homeostasis resulting from the suppression of scale bone cells: the scale is a good model for the evaluation of heavy metals in bone metabolism", Journal of bone and mineral metabolism, 2004, Vol. 22 (5), p439-446.

[21] D A Blake, R C Blake, M Khosraviani, et al. "Immunoassays for metal ions", Analytica chimica acta, 1998, Vol. 376 (1), p13-19.

[22] D A Blake, R M Jones, R C Blake, et al. "Antibody-based sensors for heavy metal ions", Biosensors and Bioelectronics, 2001, Vol. 16 (9), p799-809.

[23] D A Blake, A R Pavlov, H Yu, et al. "Antibodies and antibody-based assays for hexavalent uranium", Analytica chimica acta, 2001, Vol. 444 (1), p3-11.

[24] D T Reardan, C F Meares, D A Goodwin, et al. "Antibodies against metal chelates", Nature. 1985, Vol. 316 (18), p265-268.

[25] R M Jones, H Yu, J B Delehanty, et al. "Monoclonal antibodies that recognize minimal differences in the three-dimensional structures of metal-chelate complexes", Bioconjugate chemistry, 2002, Vol. 13 (3), p408-415.

[26] M Khosraviani, R C Blake, A R Pavlov, et al. "Binding properties of a monoclonal antibody directed toward lead-chelate complexes", Bioconjugate Chemistry, 2000, Vol. 11 (2), p267-277.

[27] G Aragay, J Pons, A Merkoci. "Recent trends in macro-, micro-, and nanomaterial- based tools and strategies for heavy-metal detection", Chemical Reviews, 2011, Vol. 111 (5), p3433-3458.

[28] G Tang, W Zhao, S Zhang. "Detection of heavy metals based on the QCM technology", China Hi-tech Enterprises, 2010, Vol. 2, p3-74.

[29] Y Zhang, W Ren, M Li. "Detection of lead and cadmium in water samples using synchronous plating bismuth fil on MWCNTs-IL-LSS composite modified electrode", Chemical Research and Application, 2013, Vol. 25 (2), p216-219.

[30] J Fei, J Li, F Yi. "Determination of trace lead by adsorptive stripping voltammetry at quercetin modified carbon paste electrode", Chinese Journal of Analytical Chemistry, 2001, Vol. 29 (8), p916-918.

[31] Y Ma, Y Sheng, C Tang, et al. "Indenofluorene based water soluble conjugated oligomers

for Hg^{2+} detection", Sensors and Actuators B: Chemical, 2013, Vol. 176 (2), p132-140.