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COLORIMETRIC DETECTION OF ARSENIC(III) IN AQUEOUS SOLUTION BASED ON APTAMER

Xijia Yang[1, 2], Dan Zhang[1, 2], Dimitar Terziev[3], Ralitsa Terziyska[3], Dongwei Zhang[1, 2], Yang Liu[1, 2], Xunfeng Chen[1, 2], Pei Zhou[*1, 2]

[1]School of Agriculture and Biology, and Bor S. Luh Food Safety Research Center, Shanghai Jiao Tong University, Shanghai, China
[2]Key Laboratory of Urban Agriculture, Ministry of Agriculture and Rural Affairs of the People's Republic of China
[3]University of National and World Economy, Bulgaria

*E-mail:zhoupei@sjtu.edu.cn

Abstract

Inorganic arsenic is considered to be the most toxic form of the element and exists in groundwater and surface water, which has threatened human health. Based on aptamer's superior biologic characteristics, three colourimetric methods were established to satisfy the demands of rapid and on-site detection. All sensing methods exhibited high selectivity for arsenic(III), and the detecting limits were even down to 5.3, 0.6 and 6ppb respectively, indicating a promising future in environmental detection of arsenic(III).

Keywords: aptamer, arsenic(III), colourimetric detection, water quality.

INTRODUCTION

Arsenic contamination in drinking water and groundwater is becoming a serious worldwide threat to human health (Cullet et al., 1986). It is estimated that about 140 million people worldwide may have been exposed to drinking water with arsenic contamination levels higher than the World Health Organizations (WHO) guideline of 10 ppb (Kalluri et al., 2009). Although arsenic exists in many different chemical forms in nature, it is found almost exclusively as arsenite (As(III) as H_3AsO_3) and arsenate (As(V) as H_3AsO_4) in water (Ensafi, Ring, Fritsch, 2010). As(III) was identified as one of the most harmful substances in the water to human health, and it is 60 times more toxic than As(V) or organic arsenic compounds. Drinking water contaminated with As(III) is associated with a number of diseases such as skin damage or problems with circulatory systems, and a high risk of getting cancer (Cullet et al., 1986). Currently, the popular methods for arsenic detection are chromatography, spectroscopic and electrochemistry methods (Mays and Hussam, 2009). These methods tend to be complex, expensive and time-consuming, so they are neither readily available in developing countries nor capable of on-site field detection. Despite that the development of ultra-sensitive assays for the real-time detection of arsenic has attracted considerable attention in recent years (Kalluri et al., 2009; Morita and Kaneko, 2006; Mulvihill et al., 2008), most of them usually needed complicated reactions or modification, thus they may be susceptible to the surrounding conditions. Therefore, it is highly necessary to develop sensitive and cost-effective methods to detect trace As(III) in the environment.

Development of three sensitive and colourimetric biosensors for As(III) detection

Aptamers have been regarded as promising tools in environmental monitoring due to the real-time and on-site detection of heavy metals (Liu, Cao, Lu, 2009). Some aptamer-based biosensors have been developed for various metal ions detection with high sensitivity and selectivity (Xiang, Tong, Lu, 2009; Liu et al., 2007).

Kim and co-workers have in vitro selected the arsenic-binding DNA aptamer (named Ars-3) (Kim et al., 2009), using an affinity column based on the systematic evolution of ligands by an exponential enrichment (SELEX) process. Such an aptamer had the highest affinity to As(III) and As(V). Herein, three sensitive and colourimetric biosensors for As(III) detection were developed based on (A) cationic polymers (PDDA) mediated(Wu et al., 2012a) or (B) surfactant induced aggregation of gold nanoparticles (Wu et al., 2012b), and (C) Hemin-H_2O_2 catalyzed TMB (Wu et al., 2013).

The presented biosensors are rapid and cost-effective, so they can be utilized in environmental and other applications.

Poly (diallyl-dimethylammonium chloride) (PDDA), a water-soluble cationic polymer, serves dual functions: (1) it promotes the aggregation of AuNPs, and (2) hybridizes the aptamer through the electrostatic interaction. As shown in Fig. 1 (A), in the absence of As(III), the Ars-3 aptamers are free and can hybridize with PDDA to form a ‘‘duplex'' structure, thus the subsequent AuNPs cannot aggregate owing to the lack of PDDA(Wu et al., 2012a). On adding As(III), the Ars-3 aptamer is exhausted firstly due to the formation of an aptamer/As(III) complex, so that the subsequent PDDA can aggregate AuNPs leading to the remarkable change in colour from wine red to blue. The optical property of solution depends on the concentration of PDDA, which is in turn conditioned directly by the content of As(III).

Hexadecyltrimethylammonium bromide (CTAB), a kind of surfactant, displays two useful features: one lies in its positive charge, which can be employed to aggregate AuNPs, and another is that it can assemble DNAs to form supramolecule with certain nanostructures(Cheng et al., 2009; Liu and Abbott, 2010; Santhiya et al., 2009; Zhou et al., 2004). These useful properties endow CTAB with special functions in the present biosensor: it not only aggregates the AuNPs but also controls their aggregation via its competitive binding to aptamers. As shown in Fig. 1 (B), in the absence of As(III), the Ars-3 aptamers are free and can assemble with CTAB forming the supramolecule, and thus the subsequent AuNPs cannot aggregate due to the lack of CTAB (Wu et al., 2012b). On adding As(III), the Ars-3 aptamer is exhausted firstly due to the formation of the aptamer-As(III) complex, so that the following CTAB can aggregate AuNPs and leading to the remarkable signal change in absorbance and colour (Wu et al., 2012b).

Hemin is usually distributed in the catalytic centre of many protein families and it can catalyze a variety of oxidation reactions like peroxidase enzymes (Zhang and Dasgupta, 1992). The primary reaction substrates for hemin or peroxidase catalysis will undergo electron transfer (ET) under redox reaction and form stable radical anions and cations, leading to the significant colour change of solutions. As shown in Fig. 1 (C), in the absence of As(III), Ars-3 aptamers are free and can bind to hemin, thus the catalytic activity will decrease due to the cover of ferric iron in the centre of hemin molecule (Wu et al., 2013). In this case, hemin has relatively lower catalytic activity and oxidizes TMB to yield the blue product of cation radical. On adding As(III), the Ars-3 aptamer is exhausted due to the formation of aptamer-As(III) complex, so that hemin can oxidize TMB thoroughly to generate the yellow product of diimine, which leads to the remarkable enhancement of absorbance value at 442 nm (Wu et al., 2013). Because the absorbance variation finally depends on the concentration of As(III), this strategy makes it possible to detect As(III) by colourimetric assay.

To achieve the best sensing performance, the experimental conditions were optimized as (A) 1.52 nM PDDA, 5 nM aptamer and MOPS buffer (pH 7.2) (Wu et al., 2012a) (B) 1.1 μM CTAB, 10 nM aptamer and MOPS buffer (pH 7.0) (Wu et al., 2012b), (C) 0.5 mM TMB, 0.12 mM hemin, 40 mM H_2O_2, 70 nM aptamer and HEPES buffer (pH 7.2) (Wu et al., 2013).

For the sensitivity study, the sensor solutions were treated with varying concentration of As(III), and the absorbance values at different wavelength were recorded. The results were shown in Fig. 2. As shown in Fig. 2A (Wu et al., 2012a), the colour of PDDA induced aggregation of AuNPs changed gradually from wine red, purple, to blue with the increasing concentration of As(III), Accordingly, the A650 value increased significantly when As(III) concentrations are greater than 1500 ppb. Once the concentration of As(III) is over 3000 ppb, some aggregated AuNPs would precipitate quickly in the solutions, which caused a sharp decrease in A520 and A650. To quantify As(III), the A650/A520 of the sensor solutions were plotted, and the A650/A520 at low As(III) concentrations were fitted to linear with a correlation coefficient of 0.996 (Table 1). The results of CTAB induced aggregation of AuNPs were similar with PDDA induced aggregation of AuNPs. As shown in Fig. 2B (Wu et al., 2012b), AuNPs aggregated gradually with increasing concentrations of As(III), and the colour of the sensing solutions changed gradually from wine red, purple, blue, to grey. When the concentration of As(III) was over 40 ppb, the colour of the sensing solution could be distinguished by the naked eye from that of the blank sample. To quantify the detection limit of the biosensor, absorbance values (ΔA) at 520 nm and 650 nm were plotted It can be observed that the absorbance had changed slightly when the concentrations of As(III) were over 400 ppb in the sensing solutions, which indicated that the binding interaction between the aptamer and As(III) had reached a balance and saturation. The ΔA at low As(III) concentrations were fitted to a linear expression (shown in Table 1). To evaluate the sensitivity of hemin-H_2O_2 system, different concentrations of As(III) were added into the sensing solutions, and the absorbance values at 442 nm were recorded. The change in colour and results were shown in Fig. 2C (Wu et al., 2013): the colour of sensing solutions also changed gradually from greenish blue, green, to greenish yellow and

the absorbance at 442 nm increased gradually with the increasing concentrations of As(III). At the same time, when the concentration of As(III) was over 50 ppb, the colour of the sensing solution could be distinguished by naked eyes from that of a blank sample. Once the concentration of As(III) exceeded 500 ppb, the colour of sensing solution had changed to greenish yellow, which also could be distinguished by naked eyes from those samples at a lower level of As(III). To quantify the detection limit of the method, the variation of absorbance values (ΔA) at 442 nm was plotted. The ΔA at low As(III) concentrations was fitted to linear (Table 1). Based on the previous report (Liu et al., 2007; Wu et al., 2011), 3 s/slope was used to determine the detection limit of the biosensor and the results are shown in Table 1. The detection limits are lower than the US EPA and WHO defined toxicity level of arsenic in drinking water (10 ppb). The results indicate that this biosensor can be potentially used to detect As(III) in aqueous solution with high sensitivity.

The selectivity of the biosensors for As(III) detection was investigated. A variety of competitive metal ions, including (A)As(V), Pb(II), Cd(II), Hg(II), Ag(I), Mg(II), Zn(II), Mn(II), Ni(II), Cu(II), Fe(II), Fe(III) and Ca(II), (B) As(V), Pb(II), Cd(II), Hg(II), Ag(I), Mg(II), Zn(II), Mn(II), Ni(II), Cu(II), Fe(II) and Ca(II), (C) As(V), Pb(II), Cd(II), Hg(II), Ag(I), Mg(II), Zn(II), Mn(II), Ni(II), Cu(II), Fe(II), Fe(III) and Ca(II), were individually added to the sensing solutions, and the variations of absorbance values (ΔA) were calculated(Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013). Fig.3 showed the difference in the colour and ΔA between blank and solutions containing (A)2000 ppb, (B)1000 ppb and (C)200 ppb of As(III) and other metal ions (Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013). The results demonstrated that all of the metal ions display slight and negligible interferences on the biosensor for As(III) detection. The interference from As(V) may be attributed to its affinity to the aptamer. As(III) and As(V) contain three hydroxy groups (–OH) in aqueous solution, which makes them easy to bind to an aptamer through the strong hydrogen bonds between –OH and amine groups (–NH or $–NH_2$) of bases in DNAs. Compared to As(III), As(V) has an additional ketone group (=O), thus it may disturb the formation of hydrogen bonds and prevent As(V) from binding to aptamer to some extent.

CONCLUSIONS

In conclusion, three colourimetric biosensors with high sensitivity and specificity for As(III) detection have been successfully developed. The principles of the biosensors were based on the aggregation of AuNPs controlled by the interaction among (A) PDDA, aptamer and As(III) or (B) aptamer, CTAB and As(III), and (C) the regulation of hemin catalytic activity by the affinity interaction between As(III) and its aptamers(Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013). According to the remarkable changes in colour before and after the introduction of As(III), the concentrations of metal ions are able to be quantified as low as 5.3, 0.6 and 6 ppb respectively, with high selectivity against other competitive ions(Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013). The experimental results reported here will offer the possibility of simple, highly sensitive and selective monitoring methods for As (III) in environmental and other applications.

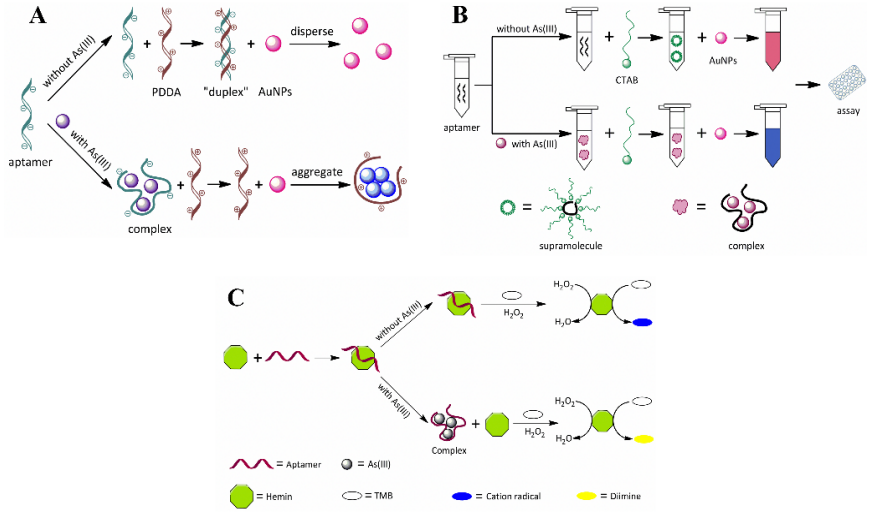

Fig. 1. *Schematic description of biosensors for colourimetric detection of As(III) (Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013)*

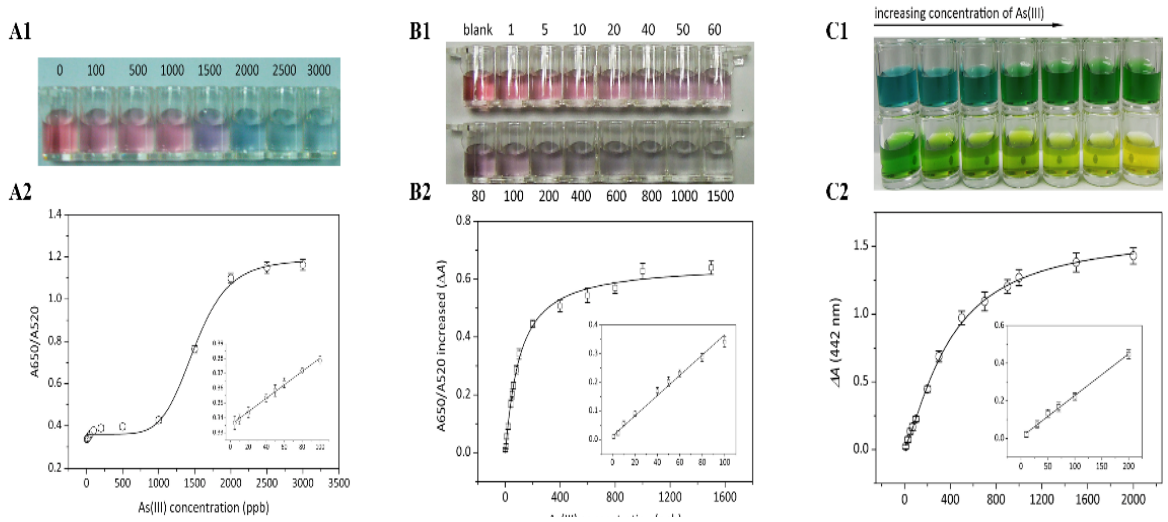

Fig. 2. *The sensitivity of the biosensor for As(III) detection using A PDDA to aggregate AuNPs, B CTAB to aggregate AuNPs and (C) hemin-H_2O_2 catalyzed TMB (Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013)*

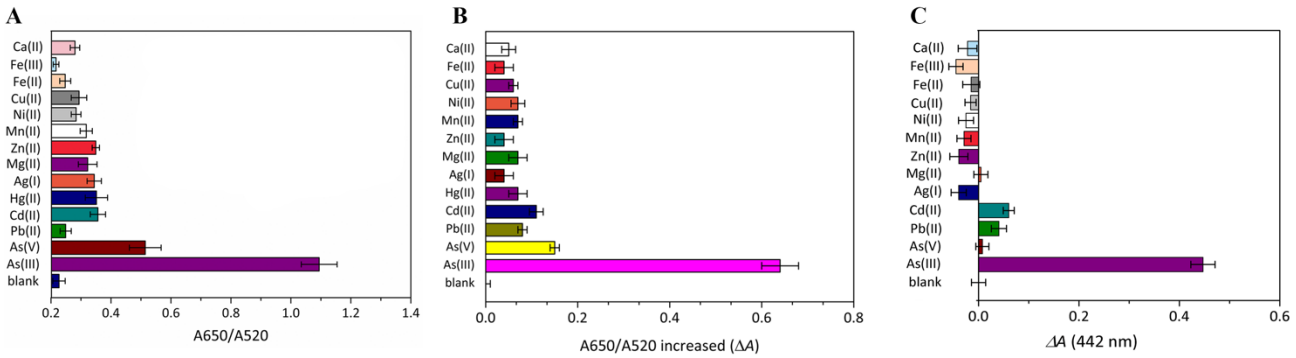

Fig. 3. *The selectivity of the biosensors forAs(III) detection. The concentrations of metal ions were (A) 2000 ppb, (B) 1000 ppb and (C) 200 ppb, respectively (Wu et al., 2012a; Wu et al., 2012b; Wu et al., 2013)*

Table 1. The sensitivity of the proposed methods

No.	Method	Fitted linear	Linear range (ppb)	LOD (ppb)	R
A	PDDA induced aggregation of AuNPs	$A=4.019\times10^{-4}$ CAs(III)+0.3351	5-100	5.3	0.996
B	CTAB induced aggregation of AuNPs	$\Delta A=3.54\times10^{-3}$ CAs(III)+0.012	1-100	0.6	0.996
C	Hemin-H_2O_2 catalyzed TMB	$\Delta A=2.2\times10^{-3}$ CAs(III)+0.007	10-200	6	0.995

LOD: Limit of detection; R: linear range.

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