Multicolor Aptasensor Based on DNA-Induced Au−Ag Nanorods for Simultaneous and Visual Detection of Inorganic and Organic Mercury

Zhiqiang Chen,† Peipei Li,† Xian Cheng,† Weijuan Yang,‡ Yongning Wu,§ Qingai Chen,∥ and FengFu Fu*†

†Key Laboratory for Analytical Science of Food Safety and Biology of MOE, Fujian Provincial Key Lab of Analysis and Detection for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China
‡State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China
§China National Center for Food Safety Risk Assessment, Beijing 100022, China
∥Department of Tourism and Hotel Management, Fujian Business University, Fuzhou 350012, P. R. China.

Supporting Information

ABSTRACT: Compared to inorganic mercury (Hg^{2+}), methyl-mercury (CH_{3}Hg^+) and ethyl-mercury (C_{2}H_{5}Hg^+) (organic mercury) not only have a much stronger toxicity but also are more easily accumulated by marine organisms to produce bioamplification. Therefore, the simultaneously onsite detection of Hg^{2+} and organic mercury is of great significance to ensure the safety of seafood, and it is also a hard challenge. We designed a T-rich aptamer, H_{7T}, for specifically recognizing Hg^{2+} and organic mercury and developed a multicolor aptasensor for simultaneous discrimination and detection of Hg^{2+} and organic mercury with only bare-eye observation using H_{7T} as a recognition probe and gold nanorods (AuNRs) as a signal. In the presence of Hg^{2+} and Ag^{+}, Hg^{2+} preferentially and specifically bind with H_{7T} immobilized on AuNRs surface and induce the formation of a monolayer Ag/Hg amalgam on the AuNRs surface after reduction, resulting in a change in color from orange to faint purple and a corresponding shift in the absorption peak from 820 to 730 nm in the solution. However, in the presence of CH_{3}Hg^{+} or C_{2}H_{5}Hg^{+} and Ag^{+}, CH_{3}Hg^{+} or C_{2}H_{5}Hg^{+} preferentially bind with H_{7T} immobilized on the AuNRs surface and induce the formation of a monolayer Ag_{0} on the AuNRs surface after reduction, which results in the change in color from orange to atrovirens and the corresponding shift in the absorption peak shift from 820 to 670 nm in the solution. Thus, the inorganic and organic mercury (total of CH_{3}Hg^{+} and C_{2}H_{5}Hg^{+}) can be specifically discriminated and detected by only bare-eye observation. The method can be used to simultaneously detect inorganic and organic mercury in seawater by the bare-eye observation with a visual detection limit of 2.0 ppm for Hg^{2+} and 10.0 ppm for organic mercury. The success of this study is a useful enlightenment to develop an instrument-free method for an onsite detection of trace inorganic and organic mercury in environment by a bare-eye observation, although the sensitivity of the method is relatively low.

1. INTRODUCTION

According to one up-to-date assessment, nearly 5207 t of mercury from anthropogenic and natural sources is released into the global atmosphere per year.¹ As one of the most toxic heavy metals, mercury can cause irreversible damage to human health and ecosystem; therefore, it is one of the most studied pollutant.² There are various mercury species existing in the environment, since inorganic or elemental mercury released into the environment can be converted into organic mercury (mainly methyl-mercury (CH_{3}Hg^+) and ethyl-mercury (C_{2}H_{5}Hg^+))² and the chemical species of mercury controls its toxicity, persistence, and migration. In comparison with inorganic mercury (Hg^{2+}), organic mercury, especially methyl-mercury (CH_{3}Hg^+) and ethyl-mercury (C_{2}H_{5}Hg^+), not only has a much stronger toxicity but also can be more easily accumulated by marine organisms to cause serious bioconcentration and biomagnification.²⁻⁴ Therefore, many countries and organizations have set a much stricter concentration limitation in the surface/marine water and aquatic product for organic mercury than that for inorganic mercury.²,⁵,⁶

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In the light of drastic toxicity of organic mercury, plenty of methods have been developed to analyze different mercury species including CH₃Hg⁺ and C₂H₅Hg⁺. Currently, the hyphenation of efficient separation technology and sensitive element-selective detectors plays a major role in mercury speciation analysis. For example, high-performance liquid chromatography or capillary electrophoresis is coupled with atomic fluorescence spectrometry or inductively coupled plasma mass spectrometry.⁷⁻¹³ However, all hyphenated techniques required costly and sophisticated apparatus, a longer analysis time, and a skillful technician; thus, they could not be used for a rapid and cost-effective onsite detection of organic mercury. To satisfy the requirement of the onsite detection of mercury, a variety of methods including colorimetric sensors, fluorescent sensors, and electrochemical sensors have been developed using a DNA aptamer or organic molecules as a recognition molecule.¹⁴⁻²⁴ However, most previous methods can only detect Hg²⁺ and are incompetent for the analysis of more toxic organic mercury such as CH₃Hg⁺ and C₂H₅Hg⁺. Lately, a turn-off fluorescent sensor was developed for a rapid and specific detection of CH₃Hg⁺ by delicately designing a dye-labeled T-rich DNA sequence for CH₃Hg⁺ recognition.²⁵,²⁶ However, the fluorescence quenching of dye-labeled DNA is more susceptible to sample matrix; therefore, the fluorescent sensor has a poorer reproducibility. In a previous study, we devised two T-rich aptamers to specifically discriminate Hg²⁺, CH₃Hg⁺ and C₂H₅Hg⁺, and developed a colorimetric sensor for the visual detection of Hg²⁺ and organic mercury (CH₃Hg⁺ and C₂H₅Hg⁺) using DNA-templated alloy Ag–Au nanoparticles as the signal.²⁸ However, the sensor needs two aptamers and presents only a single color, which seriously confine the accuracy of visual inspection. Gold nanorods (AuNRs) have a broadly tunable aspect ratio (ratio of length-to-diameter)²⁸⁻³⁰ thus, AuNRs suspended in solution may display a range of colors and surface plasmon resonance absorption. In view of the above optical properties, AuNRs have been commonly used for fabricating multicolor colorimetric sensors for the visual detection of various targets such as glucose, antigen, and ions.²⁵,⁵¹⁻⁵⁴ In this study, we developed a multicolor colorimetric method for the specific discrimination and detection of organic mercury (total of CH₃Hg⁺ and C₂H₅Hg⁺) and inorganic mercury (Hg²⁺) via only bare-eye observation based on specific T-rich DNA and DNA-induced Ag–Au AuNRs to provide a facile method for a cost-effective, rapid, and specific detection of organic mercury in surface/marine water and aquatic product to prevent harm from mercury.

2. RESULTS AND DISCUSSION

2.1. Experimental Principle for Multicolor Visual Detection of Inorganic and Organic Mercury. It has been reported that the T-rich DNA sequence has a higher binding affinity to mercury species, and the binding affinity generally increased in the order of Hg²⁺ < CH₃Hg⁺ ~ C₂H₅Hg⁺.²⁵,²⁷,³¹ Importantly, the binding affinity of the T-rich DNA sequence to different mercury species can be tuned by controlling the numbers and location of T bases.³¹ In addition, DNA molecules also have binding affinity to transition-metal ions such as Ag⁺ and Au³⁺, and thus was usually employed as a scaffold for Ag⁺ and Au³⁺ to produce DNA-templated Ag or Au nanoparticles.³⁵ However, the binding affinity of the T-rich DNA to Ag and Au is much lower than to mercury species.³¹ Thus, by referencing previous organic mercury and inorganic mercury and developed a multicolor colorimetric method for simultaneous and visual detection of organic mercury and inorganic mercury using Au–Ag alloy nanorods as a signal. As shown in Scheme 1, in the presence of only Ag⁺ (without mercury species), AuNRs keep their original aspect ratio since Ag⁺ in solution was bound by controlling the numbers and location of T bases. In this study, we designed a T-rich aptamer (HT7; see Table S1 in the Supporting Information, SI) for specifically discriminating organic mercury and inorganic mercury using Au–Ag alloy nanorods as a signal.
keeps Ag⁺ in the solution since the aptamer has much stronger binding affinity to CH₃Hg⁺ or C₂H₅Hg⁺ than to Ag⁺. After reducing with NaBH₄, the Ag⁺ in the solution were reduced to Ag⁰ on the AuNRs surface, which obviously changes the aspect ratio of AuNRs and thus makes the solution show atrovirens. This provided a multicolor platform for a simultaneous and instrument-free visual discrimination and detection of organic mercury (total of CH₃Hg⁺ and C₂H₅Hg⁺) and inorganic mercury (Hg₂⁺).

To verify the feasibility of our strategy, the color change and the UV-vis absorption spectra of the assay system were investigated under different conditions. According to the results shown in Figure 1, in the absence of mercury species, the assay system shows an orange color (AuNRs’ original color) and its spectrum has the highest absorption at 820 nm (curve a), indicating that the aspect ratio of AuNRs does not change since the Ag⁺ are not reduced to Ag⁰ on the AuNRs surface. In the case of Hg₂⁺, the assay system showed a faint purple color and a correspondingly biggest absorption peak shift to 730 nm (Figure 1b), suggesting that the aspect ratio of AuNRs becomes small since a thin layer of Ag/Hg amalgam was formed on the AuNRs surface. Finally, in the presence of CH₃Hg⁺ or C₂H₅Hg⁺, the color of the solution changed to atrovirens and the biggest absorption peak shifted to 670 nm (Figure 1c,d). This is because CH₃Hg⁺ or C₂H₅Hg⁺ preferentially bind with DNA and cause the Ag⁺ in the solution to be reduced to Ag⁰ on the AuNRs surface to form Au–Ag nanorods, which obviously changes the aspect ratio of AuNRs.

The transmission electron microscopy (TEM) images (Figure 2a) further confirmed our above deduction. As shown in Figure 2, in the absence of mercury species, AuNRs have a larger aspect ratio and only little Ag is detected in the EDX mapping image (Figure 2a) because the Ag⁺ are bound by H₁₇DNA, and thus were not reduced to Ag⁰ on the AuNRs surface. In the presence of Hg₂⁺, AuNRs were observed to have a slightly bigger width and contained a small amount of Hg and Ag (Figure 2b), indicating that Hg₂⁺ was preferentially bound on H₁₇DNA and then reduced to Hg⁰ to form the Ag/Hg amalgamation on the AuNRs surface. In contrast, in the case of CH₃Hg⁺ or C₂H₅Hg⁺, AuNRs were observed to have the smallest aspect ratio and contain obvious Ag and Hg (Figure 2c,d), indicating the formation of Ag–Au alloy nanorods after reduction since DNA preferentially binds with CH₃Hg⁺ or C₂H₅Hg⁺ and keeps Ag⁺ as a free ion in the solution.

2.2. Optimization of T-Rich DNA Sequence. As we mentioned above, T-rich DNA has a high binding affinity to mercury species. In addition, its binding affinity is different for different mercury species and can be tuned by altering the T base numbers and locations. To realize the specific recognition of organic mercury and inorganic mercury, four different DNA sequences including one random sequence (H₁₇) and three T-rich sequences (H₁₇₁₉, H₁₇₇, and H₁₇₉) (Table S1 in SI) were designed rationaly according to the principles of metallic ion-mediated stable base pairs.

2.3. Optimization of Other Important Experimental Conditions. After choosing H₁₇ as the optimal DNA sequence, other important experimental conditions including H₁₇ aptamer concentration, reducing agent, Ag⁺ concentration, pH of buffer, and so on were also optimized in detail to obtain the best analytical performance.

To obtain the optimum concentration of the H₁₇ aptamer, we selected several concentrations of the H₁₇ aptamer (15, 20, and 25 μM) for further optimization. The results are shown in Figure S1 in the SI. Under low aptamer concentration (15 μM) or high aptamer concentration (25 μM), the Hg⁰⁺ and CH₃Hg⁺ or C₂H₅Hg⁺ cannot be clearly discriminated and detected by the solution color. This may be attributed to the fact that excess H₁₇ will bind both the mercury species and Ag⁺, whereas insufficient H₁₇ cannot completely bind the mercury species. Only when the H₁₇ concentration is 20 μM, the Hg⁰⁺ and CH₃Hg⁺ or C₂H₅Hg⁺ can be clearly differentiated by the solution color; thus, 20 μM of H₁₇ aptamer was regarded as the optimal selection and used for next operation.

The reducing agent used to reduce Ag⁺ to Ag⁰ is another key factor in this experiment. To explore the effect of different reductants on the analytical performance, NaBH₄, trisodium citrate, and ascorbic acid (AA) were used as reductants in the experiment. Results shown in Figure S2 (see the SI) indicate that Hg⁰⁺ and CH₃Hg⁺ or C₂H₅Hg⁺ show approximately the same color and similar absorption spectrum when trisodium citrate and ascorbic acid were used as reductants; thus, inorganic mercury and organic mercury cannot be discriminated and detected visually. This finding may be attributed to the fact that the reducibilities of trisodium citrate and AA is lower than that of NaBH₄ and thus Ag⁺ cannot be rapidly reduced to form Ag⁰ on the AuNRs surface. Only NaBH₄ can make a notable distinction in the solution color between Hg⁰⁺ and organic mercury. Therefore, NaBH₄ was used to reduce Ag⁺ to Ag⁰ in this study. To obtain the optimal NaBH₄ concentration, four different NaBH₄ concentrations (5, 70, 85, and 100 mM) were investigated in our experiment. As shown in the results in Figure S3 (see the SI), 70 mM of
NaBH$_4$ can lead to the most notable distinction in the solution color and UV−vis absorption spectra between the Hg$^{2+}$ and organic mercury; thus, 70 mM of NaBH$_4$ solution was used in the study.

In our assay, Ag$^+$ ions were reduced to Ag$^0$ to form Au−Ag nanorods and thus generated a color change. So, the concentration of Ag$^+$ will influence the analytical performance of the method. To obtain the optimal Ag$^+$ concentration, different Ag$^+$ concentrations (0.5, 1.0, 2.0, and 5.0 mM) were tested. As shown in Figure S4 (see the SI), Hg$^{2+}$ and organic mercury display the most notable distinction in solution color when Ag$^+$ concentration is 1.0 mM; thus, 1.0 mM of Ag$^+$ was used in this study.

Finally, we optimized the pH of Tris−HNO$_3$ buffer solution. From the result shown in Figure S5 (see the SI), the pH of Tris−HNO$_3$ buffer influenced slightly the reducibility of
NaBH₄ and thus affected the formation of Ag–Hg nanorods. According to the result, the colors of the final solution among blank, Hg²⁺, and CH₃Hg⁺/C₂H₅Hg⁺ can be clearly discriminated via bare-eye observation when the pH is 8.0. Thus, the Tris–HNO₃ buffer with the pH of 8.0 was used in the experiment.

2.4. Selectivity of the Proposed Method. Other metallic ions such as Fe³⁺, Mg²⁺, Zn²⁺, Cu²⁺, Pb²⁺, Co²⁺, Cr³⁺, Cd²⁺, and Ni²⁺ may interfere with the detection of mercury species. To investigate the specificity of our method to mercury species, we detecting above interfering ions, Hg²⁺, CH₃Hg⁺, and C₂H₅Hg⁺, respectively, with the developed method. It can be clearly observed from Figure 4 that Hg²⁺ induced a change in color from orange to faint purple and a shift in absorption peak from 820 to 740 nm, whereas CH₃Hg⁺ and C₂H₅Hg⁺ caused a change in color from orange to atrovirens and a shift in absorption peak from 820 to 670 nm. For other ions, the solution showed the same color (orange) and absorption peak (820 nm) as that of the original AuNRs solution (blank). The above experiment indicated that the developed method has a good specificity to Hg²⁺ and organic mercury (CH₃Hg⁺ and C₂H₅Hg⁺), and other coexisting metallic ions do not interfere with the detection of mercury species.

In addition, we find that the coexistence of organic mercury does not affect the detection of inorganic mercury, whereas the presence of Hg⁺ will interfere with the detection of organic mercury. We demonstrated that the interference of Hg⁺ with organic mercury detection can be eliminated by reducing Hg⁺ to Hg²⁺ with ascorbic acid and then removing Hg²⁺ by introducing N₂ gas into the sample before organic mercury detection (Figure S6 in the SI). Thus, the inorganic mercury and organic mercury in the sample can be simultaneously detected with high specificity using our method.

2.5. Analytical Performance of the Proposed Method. Under optimal conditions, the analytical performance of the proposed method was investigated by detecting different concentrations of Hg²⁺, CH₃Hg⁺, and C₂H₅Hg⁺, respectively. As shown in Figure 5, the color of the solution changed from orange to purple in a step-by-step manner when the Hg²⁺ concentration increased from 0.0 to 20.0 ppm. The change in color can be clearly observed by bare eye when the Hg²⁺ concentration is 2.0 ppm, i.e., the visual detection limit of Hg²⁺ is 2.0 ppm. For CH₃Hg⁺ and C₂H₅Hg⁺, when the concentration increased from 0.0 to 100 ppm, the color of the final solution changed from orange to atrovirens in a step-by-step manner. The change in color can be clearly observed by the bare eye when the CH₃Hg⁺ or C₂H₅Hg⁺ concentration is 10.0 ppm, i.e., the visual detection limit of organic mercury is 10.0 ppm.

2.6. Simultaneous Detection of Inorganic and Organic Mercury in Seawater. To evaluate the reliability and practicality of our method, the concentrations of inorganic and organic mercury in seawater were detected with our method. As the results in Table 1 show, the inorganic and organic mercury in seawater can be specifically and rapidly detected with satisfactory accuracy by only bare-eye observation. Although the sensitivity of our method is relatively lower, the success of this study provided a useful enlightenment to develop an instrument-free and simple method for the onsite visual detection of trace inorganic and organic mercury in environment.

3. CONCLUSIONS

In summary, a multicolor aptasensor was designed and developed for the simultaneous discrimination and detection of inorganic mercury (Hg²⁺) and organic mercury (total CH₃Hg⁺ and C₂H₅Hg⁺) by only bare-eye observation based on DNA-induced Au–Ag nanorods. We designed a T-rich aptamer, H₇, for specifically recognizing the inorganic and organic mercury. We demonstrated that in the presence of inorganic mercury and Ag⁺, H₇ preferentially and specifically bind with H₇ immobilized on the AuNRs surface and induce the formation of a monolayer Ag/Hg amalgam on the AuNRs surface after reduction, which leads to change in color from orange to faint purple and a shift in the absorption peak from
820 to 730 nm in the solution. In the presence of CH$_3$Hg$^+$ or C$_2$H$_5$Hg$^+$ and Ag$^+$, CH$_3$Hg$^+$ or C$_2$H$_5$Hg$^+$ preferentially bind with HT7 immobilized on the AuNRs surface and induce the formation of a monolayer Ag on the AuNRs surface after reduction, which leads a change in color from orange to atrovirens and a shift in absorption peak from 820 to 670 nm in the solution. The method can be used to simultaneously detect inorganic mercury and organic mercury in the aqueous environment by bare-eye observation with a visual detection limit of 2.0 ppm for inorganic mercury and 10.0 ppm for organic mercury. The success of this study is a useful enlightenment to develop an instrument-free and simple method for the onsite detection of trace inorganic and organic mercury in environment by bare-eye observation, although the sensitivity of the method is relatively low.

4. EXPERIMENTAL SECTION

4.1. Preparation of AuNRs. Cetyl trimethylammonium bromide (CTAB)-coated AuNRs were synthesized by seed-mediated growth according to the previous method. Briefly, 10 mL of 0.1 M CTAB was mixed with 0.25 mL of 0.01 M HAuCl$_4$ in a 20 mL glass bottle with gentle mixing. Then, 0.6 mL of a freshly prepared, ice-cold 0.01 M NaBH$_4$ solution was quickly added into the mixture, followed by rapid stirring for 2
min. The color of the solution changed from dark yellow to brownish yellow, indicating the seed solution formation. The seed solution was kept at room temperature for at least 2 h before use. To prepare AuNRs growth solution, 2.0 mL of 0.01 M HAuCl₄ and 0.4 mL of 0.01 M AgNO₃ aqueous solution were mixed with 40 mL of 0.1 M CTAB in a 50-mL glass bottle, then 0.8 mL of 1.0 M HCl was injected into the mixture, and the pH of the solution was adjusted to 1–2. After gently mixing the solution, 0.32 mL of 0.1 M ascorbic acid (AA) was added and the growth solution was obtained. Finally, 0.096 mL of seed solution was added into the growth solution, the mixture was gently stirred for 10 s, and then matured for 24 h under static conditions at room temperature to obtain AuNRs solution. The as-prepared AuNRs were concentrated 10 times by centrifuging at 10,000 rpm for 20 min before being used in the experiment.

4.2. Specifically Detecting Inorganic Mercury and Organic Mercury. For specifically detecting Hg²⁺, 20 μL of the above prepared AuNRs solution and 20 μL of 0.01 μM H⁷⁺, DNA solution were mixed under room temperature and the mixture was gently stirred for 2 h to immobilize DNA on AuNRs. Then, the mixture was centrifuged at 5000 rpm for 20 min and the supernatant was discarded. Subsequently, 10 μL of 1 mM Ag⁺ solution and 10 μL of Hg²⁺ standard or sample solution were added and the whole was incubated for 1 h at room temperature. Finally, 100 μL of the Tris–HNO₃ buffer (pH 8.0) and 10 μL of 70 mM NaBH₄ solution were added successively and the whole was gently mixed for 10 s and kept for 5 min under static conditions. The change in the color of the solution was observed by bare eye and recorded with a digital camera. The UV–vis absorption spectrum of the solution was measured with a microplate reader in the range of 400–1000 nm. The concentration of Hg²⁺ was quantified based on the bare-eye observation.

For specifically detecting organic mercury, 20 μL of the above prepared AuNRs solution and 20 μL of 0.01 μM H⁷⁺, DNA solution were mixed under room temperature and the mixture was gently stirred for 2 h to immobilize DNA on AuNRs. Then, the mixture was centrifuged at 5000 rpm for 20 min and the supernatant was discarded. Subsequently, 10 μL of 1 mM Ag⁺ solution and 10 μL of CH₃Hg²⁺ or C₃H₇Hg²⁺ standard or sample were added and the whole was incubated for 1 h at room temperature. Finally, 100 μL of the Tris–HNO₃ buffer (pH 8.0) and 10 μL of 70 mM NaBH₄ solution were added successively and the whole was gently mixed for 10 s and kept for 5 min under static conditions. The change in the color of the solution was observed by bare eye and recorded with a digital camera. The UV–vis absorption spectrum of the solution was measured with a microplate reader in the range of 400–1000 nm. The concentration of organic mercury was quantified based on the bare-eye observation.

4.3. Determination of Seawater Sample. Seawater samples were collected from the coastal water of Pingtan, Fujian in China. The seawater sample was filtrated through a 0.22 μm membrane filter to remove residues. The Hg²⁺ in seawater was directly detected with the above method. For detecting organic mercury in seawater, a final concentration of 50 mM ascorbic acid (AA) was added into the seawater to reduce inorganic mercury for 1 h, and then N₂ gas was gently introduced into the sample to remove elemental mercury for 5 min. Finally, the organic mercury in seawater was detected according to the above procedure. The seawater samples spiked with different concentrations of Hg²⁺ or C₃H₇Hg²⁺ or C₃H₇Hg²⁺ standards were also analyzed with the same method to obtain recovery.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01994.

Chemicals and apparatus used in the experiments; DNA sequences used in the experiment (Table S1); and optimization of other important experimental conditions (Figures S1–S6) (PDF)

AUTHOR INFORMATION

Corresponding Authors
*E-mail: kuihuadexiao@163.com (W.Y.).
*E-mail: fengfu@fzu.edu.cn (F.F.).

ORCID
FengFu Fu: 0000-0002-8823-7672

Author Contributions
F.F. and W.Y. performed the experimental design, data analysis, and interpretation. Z.C. performed the experimental details and took all photos used in figures and tables. P.L. and X.C. co-performed the experimental details. Y.W. and Q.C. co-performed the data analysis and interpretation. The manuscript was written through contributions of all authors, and all authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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