Application of chemiluminescent resonance energy transfer and graphene sheet for the rapid quantification of mercury in a sample

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ABSTRACT

Using intra chemiluminescent resonance energy transfer (CRET) between DNA aptamer and fluorescein and physical property of graphene sheet, cost-effective and simple biosensor was developed for the rapid quantification of Hg^{2+} in a sample. Relatively excess DNA aptamer-conjugated fluorescein (e.g., 100 nM) in Tris-HCl (pH 8) captured Hg^{2+} in a sample for the 15-min incubation at room temperature (21 ± 2 ℃). Graphene sheets added in the solution were incubated for 5 minutes at room temperature in order to remove free DNA aptamer remaining after the binding between DNA aptamer and Hg^{2+} based on the principle of π-π interaction between graphene sheet and free DNA aptamer. 20 µl of solution containing DNA aptamer-bound Hg^{2+} and free DNA aptamer was mixed with tetra-n-propylammonium hydroxide (10 µl) in a borosilicate test tube. Then, 3,4,5-trimethoxylphenyl-glyoxal (TMPG, 150 µl) was added in the test tube to generate strong light emitted with rapid intra CRET in the presence of Hg^{2+}. Relative CL intensity was exponentially decreased with the increase of Hg^{2+}. The limit of detection (10 ppb) for the biosensor with extremely low background was as low as those of conventional analytical methods capable of quantifying Hg^{2+}. Also, the accuracy, precision, and recovery of highly sensitive biosensor were excellent within acceptable error range.

Keywords: Graphene sheet, Chemiluminescence resonance energy transfer (CRET), Aptamer, Mercury

Introduction

Due to the virulent toxicity of $Hg^{2+,}$ various analytical methods and sensors have been developed (1). With the development of aptamers capable of rapidly capturing with outstanding specificity in a sample, for example, various biosensors have been developed with an appropriate optical detection (e.g., absorbance (2), fluorescence (3), chemiluminescence (4)).

Guanine chemiluminescence (5, 6) capable of emitting bright light from the reaction between guanine of aptamer and 3,4,5-trimethoxylphenyl-glyoxal (TMPG) began to be applied as a new optical sensor for the quantification of a specific analyte in a sample because the sensitivity of guanine chemiluminescence is better than absorbance and fluorescence detections.

We developed a cost-effective and easy-to-use biosensor for the rapid quantification of Hg2+ in this research.

Experimental

Chemical and materials

Two different types of mercury aptamer, as shown below, were purchased from Alpha DNA.

A1: 5'-fluorescein-GGGGTTCTTCCCCTTGTTCCCC-3'

A2: 5'-fluorescein-GCGCTTCTTCCCCTTGTTGCGC-3'

3,4,5-trimethoxyl phenylglyoxal hydrate (TMPG, 97 %) was purchased from Matrix Scientific (Columbia, SC, USA). $FeCl_2$ (99 %), $FeCl_3$ (99 %), Tetra-*n*-propylammonium hydroxide (TPA, 40 % w/w aqueous solution), deionized H_2O (HPLC grade) were purchased from Alfa Aesar (Ward Hill, MA, USA). N,N-Dimethylformamide (DMF) and deionized water purchased from EMD (Billerica, MA, USA). Various types of buffer solutions (pH 7.0 – 8.5) were purchased from Teknova (Holliser, CA, USA). Graphene oxide (GO) was purchased from Graphene Supermarket (Calverton, NY, USA). Graphene film was provided from Department of Sustainable Biomaterials of Virgina Tech. 7.0 mM $Hg(NO_3)_2$ stock solution was purchased from VWR.

Quantification of Hg^{2+}

A certain concentration of Hg^{2+} (100 µl) in water was mixed with mercury aptamer (1 µM, 100 µl) in PBS buffer (pH 7.4). The mixture was incubated for 15 min at room temperature (21 ± 2 ℃). After the incubation, the mixture (20 µl) was mixed with 20 mM TPA (10 µl) in a borosilicate test tube. Then the test tube was inserted into the luminometer (Lumat 9507, Beththold, Inc, Germany). Then, CL immediately emitted in the test tube with the addition of 4 mM TMPG (100 µl) through a syringe pump of luminomeer was measured for 20 seconds.

Results and Discussion

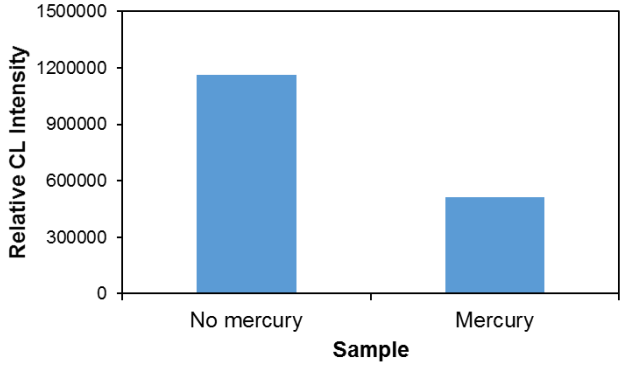

Fig. 1 Effect of mercury in guanine chemiluminescence using A1 aptamer.

As shown in Fig. 1, relative CL intensity in the absence of Hg^{2+} was higher than that in the presence of Hg^{2+}. The result indicates that (1) A1 captured Hg^{2+} and (2) A1 bound with Hg^{2+} cannot emit light.

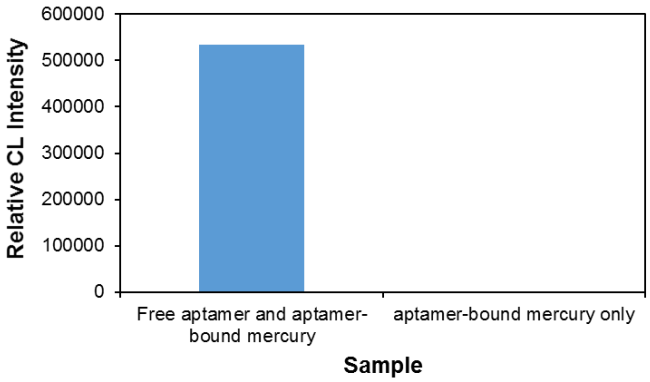

Fig. 2 Guanine chemilumescence under two different condition.

Fig. 2 is a proof that mercury aptamer-bound Hg^{2+} cannot emit lught. In order to confirm the result shown in Fig.2, free aptamer was removed using magnetic graphene oxide or graphen sheet (0.5 mm × 0.5 mm) due to the π-π interaction between free aptamer and the surface of graphene oxide (5, 6).

Based on the experimental results shown in Figs 1 and 2, we concluded that guanine of free aptamer can react with TMPG in the presence of TPA to emit bright light, whereas guanine-cytosine in the presence of Hg^{2+} because hydgen and primary amine of guanine were already interact with hydrogen and secondary amine of cytosine before adding TMPG. Fig. 3 is the possible mechanism of guanine chemiluminescence reaction in the absence and presence of Hg^{2+}.

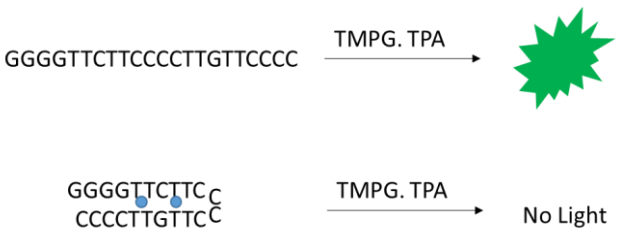

Fig. 3 Guanine chemiluminescence in the absence and presence of Hg^{2+}.

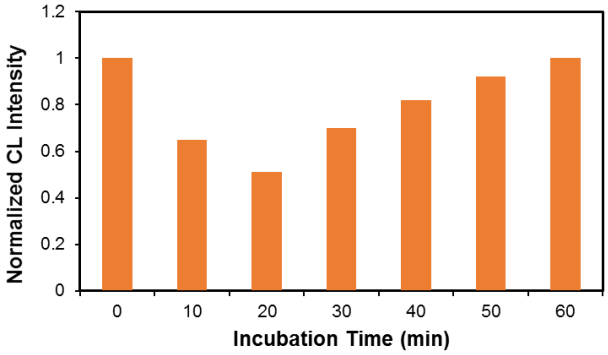

Fig. 4 Guanine chemiluminescence in the presence of Hg2+ (100 ppb) on the different incubation times for the interaction between free aptamer and Hg^{2+}.

As shown in Fig. 4, relative CL intensity of mixture containing mercury aptamer and Hg^{2+} was dependent on the incubation time to form aptamer-bound Hg^{2+}. With the increase of incubation tiem (up to 20 min), relative CL intensity was exponetially decreased. Then, relative CL intensity began to enhance with the increase of incubation time (up to 60 min). relative CL intensity measured after 60-min incubation was simliar to that observed with no incubation. The result indicates that Hg2+ in aptamer-bound Hg^{2+} began to escape after 20-min incubation. Thus, we selected 20-min incubation time for quantifying trace levels of Hg^{2+} in a sample.

Based on the preliminary experimental results shown in figs 1 ~ 4, we were able to quantify trace levels of Hg^{2+} with a wide calibration curve (10 ppb ~ 1.5 ppm). Also, the range of recovery (N = 3) of biosensor was 91 ~ 105 %. Also, accuracy and precision of biosensor were accepatable within the statistical error range (5 %).

Conclusions

We developed the cost-effective, easy-to use, and simple biosensor capable of rapidly quantifying trace levels of Hg^{2+} in a sample. We expected that novel technology reported in the paper can be applied to analyze various heavy metals in sample such as food and drinking water.

Also, it is possible that the method can be applied in chemistry, envirionmental science, and toxicology,

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