



A colorimetric assay for detection of 6-OH-BDE-47 using 6-OH-BDE-47-specific aptamers and gold nanoparticles

Un-Jung Kim^{a,1}, Byoung Chan Kim^{a,b,*}

^a Center for Environment, Health and Welfare Research, Korea Institute Science and Technology (KIST), 39-1, Hawolgok-dong, Seongbuk-gu, Seoul 02792, Republic of Korea

^b Department of Energy and Environmental Engineering, University of Science & Technology, Seoul 02792, Republic of Korea

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ABSTRACT

A colorimetric aptamer/gold nanoparticle (AuNP) sensor to detect 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE-47) was developed using AuNPs and a 6-OH-BDE-47-specific ssDNA aptamer. 6-OH-BDE-47 is mono hydroxyl-substituted form of a major congener (2,2',4,4'-tetrabromodiphenyl ether, BDE-47) of the polybrominated diphenyl ethers (PBDEs), which are representative persistent organic pollutants (POPs) and are widely used brominated flame retardants (BFR). AuNPs coated with 6-OH-BDE-47-specific aptamers initially exhibited repulsion; however, the aggregation of AuNPs was induced upon the introduction of 6-OH-BDE-47, detaching the aptamers from the AuNPs due to strong interactions between the aptamers and the target. In the colorimetric assay, the color of the testing solution changed from red to blue according to the degree of AuNP aggregation, with a limit of detection in the region of parts-per-billion (ppb), and a dynamic range of detection ranging from 5 ppb to 1 ppm. This colorimetric assay was then repeated using paper that absorbed AuNPs covered with 6-OH-BDE-47-specific aptamers. Similarly, a color change was observed when the paper was exposed to 6-OH-BDE-47. Compared to conventional trace quantitative analysis methods, this paper-based colorimetric assay is advantageous for quick screening in the case of sudden spills of highly concentrated chemicals.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) are the most famous brominated flame retardants (BFRs), with hundreds of congeners being widely used in various types of products, such as textiles, electronic devices, and office supplies. As a result, they are commonly found environmental pollutants worldwide [1,2]. Indeed, PBDEs are notorious persistent organic pollutants (POPs) due to their toxicities, persistence, and potential to be bioaccumulated in both humans and in the wider environment. Since 2009, two commercial flame retardant products containing penta- and octa-BDEs have been regulated by the Stockholm Convention. This has led

to a number of studies investigating the environmental behavior of both these chemicals, alternative compounds and the potential effects of their exposure [3–7]. To date, conventional methodology to analyze PBDEs is based on the quantification of trace amounts of PBDEs using high resolution analytical systems, such as GC/HRMS or GC/MS/MS [8,9].

Indeed, trace environmental quantitative analysis focusing on specific identification and trace level quantitation is essential to understand the fate of chemicals present in the environment, with the requirement for high throughput screening within a limited time and budget often being raised. Therefore, in circumstances where rapid screening is necessary, such as a sudden high level exposure of chemicals due to accidental leakages or acute exposure accidents at workplace, trace quantitative analysis might be unsuitable due to the time taken to carry out the analysis and the professional labor requirements needed to control the analytical process. A number of trials to determine alternative screening methods have been carried out for various micropollutants, with some of those targeting PBDEs. However, the majority of these methods employed screening based on immunoassays with synthetic antibodies, haptens, or other kinds of biological carriers

* Corresponding author at: Center for Environment, Health and Welfare Research, Korea Institute Science and Technology (KIST), 39-1, Hawolgok-dong, Seongbuk-gu, Seoul 02792, Republic of Korea.

E-mail address: bchankim@kist.re.kr (B.C. Kim).

¹ Present address: Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, USA.

[10–16]. Considering the limitations of bioassays, such as uncontrollable enzyme and antibody reactivities, in addition to the time required to perform the assays, a strong requirement still exists to develop simple, facile, and rapid screening methods combined with quantitative analysis. One potential tool to pursue this goal is the colorimetric sensor approach based on gold nanoparticles (AuNPs) conjugated with target-specific DNA oligonucleotide aptamers [17,18]. DNA oligonucleotide aptamers are synthetic DNA molecules that have been selected from an initial pool of $>10^{14}$ random molecules. They were selected by repeated binding to target compounds until a sufficiently high affinity was expressed to recognize targets over a wide concentration range, i.e., from the nanomolar (nM) to the millimolar (mM) scale [19]. The AuNP based colorimetric assay, for example, is a representative sensor platform that is already widely used in the fields of biomedical and clinical diagnosis and food safety [20,21]. Indeed, AuNP-based colorimetric assays are simple to operate, as the differentiation of AuNP interactions derived from introduction of the target of interest is clearly indicated by a change in color and, can be easily observed with the naked eye [20,22–24].

Thus, we herein optimized simple colorimetric assay and applied it to paper-based colorimetric assay aim to detect the environmentally pervasive 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) through detection of one hydroxyl group substituted form of BDE-47 as 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE-47), based on AuNPs coated with single-stranded 6-OH-BDE-47 specific DNA aptamers. BDE-47 is one of a dominant congener of PBDEs that widely found in both biological samples and abiotic environmental matrices. Based on the predominant occurrence and relative slow degradation rate of BDE-47 against other congeners, this congener was thought to be useful target isomer for the screening of PBDEs. To the best of our knowledge, this is the first study to carry out a AuNP-based colorimetric assay of PBDEs based on ssDNA aptamers and to propose its application as a tool for the rapid preliminary screening of PBDEs. We therefore aim to present a basic and fundamental methodology for the development of a direct field screening approach for diverse micropollutants, and to expand the applicability of ssDNA aptamers from their original targets of biomolecules and human disease identifiers to environmental pollutants.

2. Materials and methods

2.1. Target compound and used reagents

The target compound, namely 6-OH-BDE-47, is a mono hydroxyl-substituted form of BDE-47, and was purchased from AccuStandard (New Haven, CT, USA). All other chemical standards employed in the assay (i.e., bisphenol A (BPA) and 6-OH-BDE-99) were purchased from AccuStandard, while tetrabromobisphenol A (TBBPA) was purchased from Wellington laboratories (Guelph, ON, Canada). Bovine serum albumin (BSA) was purchased from Thermo Scientific (Walltham, MA, USA), and the gold nanoparticles (AuNPs, 10 nm diameter, optical density = 1, stabilized suspension in 0.1 mM PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cellulose filter paper (Whatman, 2.5 μ m pore size) was used to test the colorimetric assay on a paper platform. Pesticide-grade *n*-hexane, dichloromethane, acetone, and methanol, in addition to HPLC-grade water, were purchased from J.T. Baker (Center Valley, PA, USA).

2.2. Aptamer sequence information

Two characterized single-stranded oligonucleotide aptamers, namely 5'-GCA ATG GTA CGG TAC TTC CGA CAG CCG GGG CAT

CAG AGC AGC CGA TTG TCT GTT GTG CCC AAA AGT GCA CGC TAC TTT GCT AA-3' (BDE-A-8) and 5'-GCA ATG GTA CGG TAC TTC CAT TGC ACG TCT CCG CCG CTT GGG TGG AGA GGC TAT TCG GCC AAA AGT GCA CGC TAC TTT GCT AA-3' (BDE-A-12), which specifically bind to 6-OH-BDE-47, were isolated and characterized as reported in our previous study [25]. In this case those two ssDNA aptamers were synthesized and purified via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Genotech, Daejeon, Korea). A solution of the resulting ssDNA aptamers in distilled water (200 nM) was then prepared, and dispensed into individual 100 μ L tubes prior to storage at -20°C until required. Immediately before use, each tube was thawed at room temperature ($20\text{--}25^{\circ}\text{C}$) over 30 min and stored on ice. All used ssDNA aptamers were disposed to avoid any deterioration in reactivity.

2.3. Colorimetric assay experimental condition optimization

The colorimetric assay was performed based on previous literature reports with minor modifications [20,22,26]. A portion of the aptamer solution dissolved in distilled water (100 μ L, 200 nM) was thawed over ice for 20 min and gently vortexed to obtain a homogenous mixture. The aptamer solution was then mixed with a portion of the AuNP solution (750 μ L, 70 nM) in the same tube. The resulting mixture was allowed to react for 30 min at 30°C using a thermomixer (Eppendorf, Hamburg, Germany) set at 700 rpm. Subsequently, the AuNP/aptamer mixture was gently vortexed for 30 s and dispensed in volumes of 275 μ L into three separate tubes. A portion of each test solution (25 μ L) was added to the tubes containing the AuNP/aptamer mixtures and incubated for 15 min at 30°C with gentle mixing (300 rpm). A 0.1 M NaCl buffer solution (200 μ L, final concentration 0.04 M NaCl) was then added slowly to the mixture to activate the interparticle AuNP repulsion. The color of the mixture was easily observed by the naked eye upon completion of the reaction for comparison. UV–vis absorption spectra (UV-2500PC, Shimadzu, Japan) were measured between 450 and 750 nm (absorption maximum of AuNPs = 510–525 nm) after transferring the prepared solution into a cuvette. Assay conditions were optimized by varying the buffer pH, reaction time, temperature, mixing conditions, and aptamer usage (i.e., individual or mixed).

2.4. Colorimetric assay using paper discs

The filter papers were washed sequentially with HPLC water, acetone, *n*-hexane, and dichloromethane three times and were dried in a fume hood prior to performing the paper disc colorimetric assays. The dried paper was then cut using a hole punch to produce discs that fit into the 96-well white-bottomed immunoassay plate. The hole punch had previously been washed with the same organic solvent mixture to prevent contamination. The discs were stored covered by hexane-rinsed aluminum foil and placed in an air-tight desiccator until use. Immediately prior to use, each paper disc was placed into a well and submerged into the previously prepared AuNP/aptamer solution (80 μ L) and dried for 15 min at 60°C . The AuNP/aptamer-containing paper disc was then exposed to specified concentrations (1–2.5 ppm) of the target BDE, and the mixture activated using the NaCl buffer solution (0.1 M). After 10 min, the color of each disc was recorded by taking a photographic image. The color difference between the control, low (1 ppm), and high (2.5 ppm) 6-OH-BDE-47 concentrations were compared using the naked eye.

2.5. Quality assurance and quality control

Procedural and fabrication blanks were included with every processing batch to demonstrate that there was no significant background contamination. Each experimental batch was conducted

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