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Inhibitor effects on molecular beacon-based mercury assays for tuning of detection range

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ABSTRACT

A series of inhibitors were incorporated to modulate the detection range of mercury assay. A single-strand DNA-based mercury probe (ssDNA-Hg) containing 5 thymine (T) bases at both termini, which forms a hairpin structure in the presence of mercuric (Hg^{2+}) ions via the T···Hg^{2+}···T interaction, was designed. Although many previous studies focused on mercury detection to improve the sensitivity and selectivity, few studies have been reported to tune the detection range to cover a wide range of target concentrations in different environments. In the present study, a new Hg^{2+} detection scheme was designed to extend, narrow and shift the range of mercury detection using inhibitors. The complementary DNA (ssDNA-C) and cationic conjugated polyelectrolyte (CPE) induced a shift or broadening of the spectral response range by functioning as an allosteric inhibitor. Iodide and cyanide ions worked as efficient depletants, adjusting the binding constant of ssDNA-Hg and Hg^{2+} ($1.1 \times 10^7\ M^{-1}$) by ~10 times (up to $\sim 1.4 \times 10^6\ M^{-1}$) in the presence of I^- or CN^- ions, resulting in a large shift in the detection range according to [I^-] or [CN^-] with a sharp titration curve via a sequestration mechanism. Similar Hg^{2+} detection range shift was successfully demonstrated using same series of inhibitors in real samples such as Han River and tap water. This study suggests that the binding affinity of probe can be tuned toward a target and the resulting detection response range can be controlled using a range of inhibitors.

1. Introduction

Mercury is highly toxic and considered one of the most hazardous pollutants because it accumulates in the vital organs and tissues of organisms throughout the food chain, resulting in irreversible cellular malfunctions with serious human health problems even at very low concentrations. Moreover, mercury has been reported to damage DNA, disrupt the immune system homeostasis and lead to death [1–3]. Therefore, a range of methodologies have been suggested to detect mercury contamination in drinking water, food, air, and soil with high sensitivity using crown ether [4,5], rhodamine [6–8], naphthalimide [9,10], as well as oligonucleotides [11–19] and their derivatives as a mercury recognition moiety.

Akira Ono reported the simple and highly selective detection of Hg^{2+} in aqueous media based on a single-stranded deoxyribonucleic acid probe containing thymine groups at both strand ends with 6-carboxyfluorescein (6-FAM) and 4-(4′-dimethylaminophenylazo)benzoic acid (DABCYL) as the fluorophore and quencher, respectively. In the presence of Hg^{2+} ions, the probe forms a hairpin structure via T···Hg^{2+}···T bond formation with a concomitant decrease in photoluminescence (PL) intensity with increasing [Hg^{2+}] (limit of detection, LOD = ~40 nM) due to close contact of the fluorophore and quencher [11]. In addition to Ono's mercury assay, a range of Hg^{2+} detection schemes have been demonstrated, showing a lower LOD than the maximum contamination level (~10 nM) defined by the US Environmental Protection Agency (EPA) [20]. Although previous studies focused on improving the sensitivity and selectivity in mercury sensory systems [21–23], few studies have attempted to tune the detection range [24,25]. Because the fixed detection range of sensors shows the lack of detection when the analyte concentration is out of detection range, new schemes to extend and/or shift the sensing range are necessarily required. For example, concentration of adenosine triphosphate (ATP) in living systems varies depending on the organelle and environment (typically 1–10 mM in cells and ~1 μM in blood) [26,27]. The lower concentration level (~nM) of human plasma ATP was

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also reported. A limited detection range complicates, or in some case precludes the general use of assays in many real applications. In addition, sensory systems responding toward higher concentrations and/or broad concentration range of analytes are also required [28]. By expanding the detection range to cover a broad range of analyte concentrations, on-site and quick preliminary screening test is possible prior to accurate titration of mercury ions. In contrary, a strategy to narrowing the detection range is also important. By focusing on the specific narrow range of [analyte], more accurate and sensitive titration is possible with a significant signal change even with small variation in [analyte]. In the case of biomolecular systems programmed to integrate multiple inputs (such as multiple disease biomarkers), the narrow detection range can reduce the signal overlap [29,30]. To cover the wide range of target concentrations in different environments, it is essential to develop a way to shift or to extend the detection range.

Plaxco and Ricci et al. suggested interesting electrochemical and/or fluorescence-based strategies to modulate the useful detection range of DNA assays using a mutated probe, inhibitors and activators. To create the mutation of probes, they modified the number of guanine (G)···cytosine (C) bonds in the DNA probe to tune the target binding affinity, producing different dissociation constants (K_d) with the target DNA [31]. They also reported a way to alter the binding affinity between the probe and target (cocaine) using allosteric inhibition with a complementary single-stranded DNA, which hybridizes with the probe DNA [32–34]. In addition, depletant DNA, which exhibits much stronger binding affinity toward a target (relative to probe), was also used to modulate the detection range. Li et al. successfully demonstrated Hg^{2+} detection range tuning based on molecular beacon and complementary DNA with different numbers of base mismatches where thymine was incorporated in mismatched points, inducing T-Hg^{2+}-T linkage in the presence of Hg^{2+} ions. With increasing the number of mismatched sites, the higher [Hg^{2+}] is needed for structural transformation into an open-chain structure, resulting in modulation of detection range (32–1800 nM, LOD = 9.5 nM) [24]. Zhao's group also reported an interesting mercury detection strategy based on trifunctional molecular beacon (TMB)-mediated quadratic amplification (TMBQA). TMBQA-based sensory system showed ultrahigh sensitivity (200 pM), however it suffers from a very narrow detection range (0.4–20 nM) [25]. The fine-tuning of detection range is a still challenge for specific targets under different environments in a range of chemical and biological assays. To cover the wide range of target concentrations in different environments, it is essential to develop an efficient and easy way to modulate the detection range.

This paper reports a simple and useful strategy to shift, extend and narrow the detection range of mercury assays based on a single-strand oligonucleotide mercury probe (ssDNA-Hg) with 5 thymine units in both ends, which can form T···Hg^{2+}···T linkages [11,12]. A fluorophore (6-FAM) and quencher (DABCYL) were labeled at both 5′ and 3′ termini of the probe. This study investigated the inhibiting characteristics of several inhibitor agents, including single-stranded complementary DNA (ssDNA-C) and cationic polyfluorene (poly[9,9′-bis(6′′-*N*,*N*,*N*-trimethylammoniumhexyl)fluorene-alt-phenylene] dibromide, c-PFP) as an allosteric inhibitor, as well as iodide and cyanide as a depletant, to fine-modulate the detection range. The successful tuning of the mercury detection range was demonstrated, showing a shifted, extended and narrowed response range. ssDNA-C forms a duplex with ssDNA-Hg via H-bonding interactions between the complementary base pairs, which retards T···Hg^{2+}···T bond formation. Similarly, cationic polyfluorene forms an electrostatic complex with ssDNA-Hg, which interrupts the formation of hairpin structured ssDNA-Hg with mercuric ions. The cationic polymer was also used as a fluorescence resonance energy transfer (FRET) donor to 6-FAM (FRET acceptor) in the probe DNA, amplifying the FRET-induced sensory signals. The binding affinities of iodide and cyanide ions toward Hg^{2+} ions are much stronger than that of ssDNA-Hg, and the presence of these ions induced a clear shift in the detection range to a higher [Hg^{2+}] with a sharp signal transition. The detailed assay characteristics for mercury detection will be discussed and compared with this series of inhibitor agents.

2. Experimental

2.1. General

All chemicals were purchased from Aldrich Chemical Co. and used as received. High performance liquid chromatography (HPLC)-purified single-strand DNA labeled with 6-carboxyfluorescein (6-FAM) and 4-(4′-dimethylaminophenylazo)benzoic acid (DABCYL) at both termini (5′-6-FAM-TTTTTTCAGTCTGCGATTTTTA-DABCYL-3′) was used as the mercury probe (ssDNA-Hg). Two types of complementary DNAs to ssDNA-Hg were designed with the sequence, 5′-TAAAAATCGCAGACTGAAAAAA-3′ (ssDNA-C1) and 5′-TCGCAGACTG-3′ (ssDNA-C2). The cationic conjugated polyelectrolyte (CPE), poly[9,9′-bis(6′′-*N*,*N*,*N*-trimethylammoniumhexyl)fluorene-alt-phenylene] dibromide (c-PFP) was synthesized using the previously reported procedure [35–37]. The UV/vis absorption spectra were measured using a Jasco (V-630) spectrophotometer. The photoluminescence (PL) spectra were obtained on a Jasco (FP-6500) spectrofluorometer with a xenon lamp excitation source using 90° angle detection for the solution samples.

2.2. Mercury detection protocols

All PL experiments were conducted in 20 mM Tris–HCl buffer (pH = 8.0). A stock solution (1.0×10^{-5} M) of ssDNA-Hg was prepared in deionized water. Mercury detection condition was optimized by varying buffer solution, pH, DNA probe concentration and temperature, etc. The ssDNA-Hg stock solution (2 μL) was diluted in 2 mL buffer ([ssDNA-Hg] = 10 nM), and the resulting solution was used for the Hg^{2+} detection experiments. The PL spectra were measured by excitation at 490 nm after shaking the probe solution for 1 min in the presence and absence of mercuric ions. For hybridization to form a duplex between ssDNA-Hg and ssDNA-C1 (or C2), the mixture was incubated at 60 °C for 30 min (in 20 mM Tris–HCl buffer) and then allowed to cool to room temperature. The PL spectra of ssDNA-Hg/ssDNA-C were measured by changing [Hg^{2+}]. In the case of CPE as an inhibitor, c-PFP was added to 2 mL of the ssDNA-Hg solution (10 nM) in 20 mM Tris–HCl buffer to form an electrostatic complex of c-PFP/ssDNA-Hg. The FRET-induced PL spectra were measured by exciting c-PFP at 380 nm with increasing [Hg^{2+}]. To check the inhibition effect of I^- and CN^-, a mixture of ssDNA-Hg and I^- (or CN^-) in 2 mL Tris-HCl buffer was prepared by changing the concentration of inhibitors. The PL spectra were obtained by exciting 6-FAM at 490 nm with changing [Hg^{2+}].

3. Results and discussion

3.1. Mercury detection

Scheme 1 describes a detection strategy via the linear–hairpin conformational change in a mercury probe in the absence or presence of mercuric ions. The mercury probe (ssDNA-Hg) has 5 thymine groups at both 5′ and 3′-terminal sides: 5′-6-FAM-TTTTTTCAGTCTGCGATTTTTA-DABCYL-3′. Mercuric ions (Hg^{2+}) can interact with thymine to form a T···Hg^{2+}···T linkage, which is more stable than the thymine (T)-adenine (A) Watson Crick base pair. Upon the addition of mercuric ions, the conformational transfor-