Label-free sensing of pH and silver nanoparticles using an “OR” logic gate

Dik-Lung Ma,a∗, Hong-Zhang Hea, Victor Pui-Yan Maa, Daniel Shiu-Hin Chana, Ka-Ho Leunga, Hai-Jing Zhongb,c, Lihua Lua, Jean-Louis Mergynd, Chung-Hang Leungb,c

a Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China
b Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China
c State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macao SAR, China
d Univ. Bordeaux, INSERM U869, Laboratoire ARNA, Institut Européen de Chimie et Biologie, F-33600, France

HIGHLIGHTS

► A DNA logic gate for silver nanoparticles and pH has been constructed.
► A platinum(II) complex produces a luminescence response to the analyte.
► The logic gate performs “OR” operations with a switch-on luminescent output.
► The system selectively detects nanomolar silver nanoparticles in aqueous solution.

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ABSTRACT

Many natural phenomena are associated with the presence of two or more separate variables. We report here an “OR” DNA logic gate based on a luminescent platinum(II) switch-on probe for silver nanoparticles and pH, both of which may be considered putative indicators of pollution. The modulation of metal complex/double-stranded DNA complex phosphorescence by Ag+ and H+ was used to construct a simple, rapid and label-free method for the label-free detection of pH and nanomolar Ag+ ions and nanoparticles in aqueous solutions with high selectivity.

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1. Introduction

Silver (Ag+) ions and silver nanoparticles (AgNPs) have found increasing applications in consumer products such as detergents, antibacterials and wound dressings, raising concerns over their potential toxicity and bioaccumulation in our environment. Ag+ ions are assigned to the highest toxicity class in heavy metal pollution [1], while AgNPs are known to exhibit adverse effects on prokaryotes, invertebrates and fishes in the aquatic environment [2,3]. Atomic absorption and plasma emission spectroscopy methods commonly used to detect silver are often expensive and time-consuming in practice, and are unsuitable for in-field detection of heavy metal ion contamination. Although luminescent detection methods for acutely toxic heavy metal ions such as mercury and lead have been widely reported, there have been comparatively fewer reports on the detection of Ag+ ions and AgNPs using luminescent probes [4–6]. Meanwhile, acid rain formed from nitrogen oxide and sulfur dioxide pollutants represents a serious environmental problem due to their impacts to terrestrial and aquatic biological lifeforms [7].

Many phenomena in different fields are modulated by the presence of two or more independent variables. For example,
intracellular H⁺ and Na⁺ concentrations are elevated in tissues of certain tumors compared to normal tissues [8]. Renal failure is associated with enhanced levels of urea, potassium and creatinine [9]. Chemical logic gates capable of responding to multiple sensory inputs simultaneously could form the basis of “smart” detection technologies that give single, distinct outputs depending on the presence or absence of various input signals. In particular, DNA logic gates have received wide attention due to the fascinating diversity of nucleic acid structures and functions that can be potentially harnessed to perform logic operations for molecular sensors, nanocomputers or nanomachines [10]. Willner and co-workers developed a DNAzyme logic gate capable of performing AND and OR operations with UO₂²⁻ and Mg²⁺ as inputs and a colorimetric output [11]. The group of Willner also developed AND and OR logic gates for Hg²⁺ and Ag⁺ ions based on DNA-functionalized quantum dots [12]. Sugimoto and co-workers reported a G-quadruplex-based OR logic gate responding to H⁺ and K⁺ ions as inputs [13]. Wang et al. have constructed a DNAzyme-based INHIBIT logic gate for K⁺ and Pb²⁺ ions that gave a colorimetric signal as the output [14]. Our group previously reported a label-free DNA based OR logic gate for H⁺ and K⁺ ions using crystal violet (CV) as signal transducer [15]. These examples demonstrate the application of DNA logic gates for the simultaneous detection of metal or hydrogen ions, based on the cation-induced modulation of DNA structure or activity. Encouraged by these successes, we endeavored to construct a DNA logic gate that could respond simultaneously to AgNPs and H⁺ ions. To best of our knowledge, no such DNA logic gate has yet been reported. As both low pH and the presence of Ag⁺ or AgNPs may be considered as indicators of pollution, such a logic gate could find potential application as a rapid sensor for in-field estimation of water or soil quality without the need for expensive sensing devices.

Ono et al. have reported that cytosine-rich oligonucleotides could be induced to form a hairpin structure stabilized by mismatched C–Ag(1)–C base pairs, and developed a probe for aqueous Ag⁺ ions using a fluorescently-labelled cytosine-rich molecular beacon [16]. Our group has also reported label-free variations of the oligonucleotide-based approach for the detection of heavy metal ions by utilizing a luminescent transition metal complex [17,18]. Meanwhile, H⁺ ions could induce the conversion of adenine-rich single-stranded DNA into a DNA duplex, via the formation of AH⁺–H⁺A base pairs as demonstrated previously [19]. Inspired by these works, we envisaged that a label-free OR logic gate could be constructed with high sensitivity and specificity based on the Ag⁺ or H⁺ ion-induced formation of duplex DNA.

Luminescent metal complexes have been widely used for the detection of DNA and other biomolecules [20–35]. In this study, a luminescent non-toxic platinum(II) metallointercalator previously developed by our group [17,18], [Pt(C²-N°N')(4-appt)]⁺ (1, C²-N°N' = 6-phenyl-2,2'-bipyridine; 4-appt = 2-amino-4-phenylamino-6-(4-pyridyl)-1,3,5-triazine) platinum(II) complex (Fig. 1) was applied for the construction of a DNA logic gate. The present luminescent DNA “OR” logic gate offers several advantages compared to other DNA-based logic gates. (1) It does not require oligonucleotide modification, such as fluorescent labeling or linking to a signal-transducing electrode, which provides simplicity and can greatly reduce the cost of the assay. (2) Luminescent metal complexes possess a long phosphorescence lifetime, allowing the luminescent readout to be easily distinguished from endogenous fluorophores using time-resolved microscopy, which allows it to be used in highly fluorescent environments. (3) The system can be easily reset by the addition of suitable chemical reagents. We present herein the construction of a luminescent DNA-based “OR” logic gate for H⁺ and Ag⁺ ions using a platinum(II) intercalator as a signal reporter and demonstration of its application for the simultaneous detection of H⁺ and Ag⁺ ions in buffer solution.

The design of our “OR” logic gate operation is depicted in Scheme 1. The adenine and cytosine-rich oligonucleotide AC₃₀ (5'-AAAAACCCCGAAAAACCCCGAAAAACCCCG-3') would be expected to exist predominantly as a single-stranded conformation in solution in the absence of H⁺ or Ag⁺ ions. The platinum(II) complex is unable to intercalate into the single-stranded DNA, and is non-emissive due to non-radiative decay of the excited state by complex–solvent interactions [17]. The addition of either H⁺ or Ag⁺ will induce a single strand-to-duplex transition based on the formation of mismatched adenine or cytosine base pairs, respectively. We presume that this could take the form of a unimolecular hairpin or an intermolecular AC₃₀–AC₃₀ duplex (Scheme 1). Intercalation of the metal complex 1 into the duplex structure protects the [Pt(C²-N°N')] moiety from the aqueous buffer environment, suppressing non-radiative decay and enhancing phosphorescence emission. Detection of AgNPs could be achieved by treatment with H₂O₂ and H₃PO₄, promoting the oxidation disintegration of the nanoparticles. The subsequent release of the Ag⁺ ions stabilizes the duplex structure through formation of the cytosine–Ag⁺–cytosine mismatch, allowing intercalation of 1 and resulting in an emission

Scheme 1. Schematic illustration of the DNA “OR” logic gate. Addition of either input (H⁺ or Ag⁺ ions) results in intercalation of platinum(II) complex 1 producing a switch-on phosphorescence response.
enhancement. This DNA “OR” logic gate thus responds to pH or AgNP inputs with a switch-on luminescence output, based on the Ag⁺ or H⁺ ion induced structure-switching of oligonucleotide AC₃₀.

2. Materials and methods

2.1. Chemicals and materials

DNA oligomers were obtained from Tech Dragon Limited (Hong Kong). The sequence of the oligomer is: AC₃₀ = 5'-AAA-AACCCCCAAAAACCCCCAAAAACCCC-3'. Emission experiments were performed in Britton–Robinson buffer (BR-Buffer) containing 0.06 M boric acid, 0.06 M phosphoric acid, and 0.06 M acetic acid at various pH values [36–40]. All chemicals used for the synthesis of complex 1 were purchased from Sigma–Aldrich and used as received. The compounds 4-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine (4-appt) [41], 6-phenyl-2,2’-bipyridine (HC=N=N) [42], [Pt(C=N-N)Cl] [43], [Pt(C=N-N)(4-appt)]⁺ [17,18] were prepared according to previously reported procedures.

2.2. Synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized by the reduction of AgNO₃ by NaBH₄ using a modified literature procedure [44]. Briefly, 10 mL of 0.5 M AgNO₃ and 10 mL of 0.5 M sodium citrate were added into a 250 mL three-necked round bottom flask. Then, 10 mL of 10 mM NaBH₄ was added dropwise to the mixture with stirring for 10 min. The AgNPs were formed instantaneously as indicated by the change of color from colorless to yellow. The resulting solution was heated to 70 °C to destroy remaining NaBH₄ and then cooled to room temperature. The AgNPs in solution were stored at 4 °C and were used without further purification.

2.3. Physical measurements

Absorption spectra were recorded on a PerkinElmer Lambda 19 UV/vis spectrometer or a Cary 300 UV/vis spectrometer at 25 °C. Emission spectra were recorded on a SPEX Fluorolog-2 Model fluorescence spectrophotometer. The photophysical data for complex 1 have been previously reported [5]. Circular dichroism (CD) spectra were measured on a JASCO-815 spectrometer.

2.4. Gel mobility shift assay

The assay was performed using a modified literature procedure [19]. Briefly, 400 μM of AC₃₀ was incubated in the BR buffer with the indicated pH (pH = 3) at 4 °C for 12 h prior to the experiment. In parallel experiments, excess Ag⁺ (50 μM) and/or 25 μM Pt complex 1 (or 5 μM ethidium bromide as control) were added to separate solutions of AC₃₀ in BR buffer. The incubated samples were resolved on 15% polyacrylamide gel in BR buffer (pH 3) at 90 V for 120 min and visualized by ethidium bromide staining.

2.5. Emission measurement

Solutions of the platinum(II) complex 1 (10.0 μM) with AC₃₀ oligonucleotide (15 μM) were prepared in a BR-Buffer at the desired pH. AgNPs were treated by the addition of H₃PO₄ (1 μM) and H₂O₂ (1 mM) for oxidation release of Ag⁺ ions. Aliquots of a millimolar stock solution of AgNPs were then added. Emission spectra were recorded in the 480–800 nm range, after equilibration at 20.0 °C for 1 h. Excitation wavelength was set at 350 nm.

3. Results and discussion

3.1. Optimization of experimental condition

We initially investigated the effect of various parameters to optimize the experimental system, such as the concentration of AC₃₀ oligonucleotide and the concentrations of the oxidizing agents. We determined that an oligonucleotide concentration of 15 μM was ideal. For oxidative release of Ag⁺ ions from AgNPs, we treated the AgNPs with phosphoric acid–hydrogen peroxide [6] ([H₃PO₄] = 1 μM, [H₂O₂] = 1 mM) for 1 h at 20.0 °C prior to measurement. The phosphorescence profile of metal complex was not significantly affected by the addition of the oxidizing agents (data not shown). Finally, we tested the effect of varying the concentration of the platinum(II) complex. A concentration of 10 μM of 1 was found to be optimal for this study, as higher concentrations gave a higher background signal, while a lower concentration resulted in reduced sensitivity. Representative optimization results are given in the supporting information (Fig. S1).

3.2. DNA-binding properties of Pt complex 1

To investigate the conformational properties of AC₃₀ in the presence of Ag⁺ and/or H⁺, we evaluated the electrophoretic mobility of the oligonucleotide in the presence or absence of Ag⁺ ion by native polyacrylamide gel electrophoresis (PAGE). The oligonucleotide was incubated at 4 °C under the appropriate conditions for 12 h to ensure complete structural transition of the oligonucleotide, and the resulting solutions were electrophoresed on a 15% polyacrylamide gel and visualized by ethidium bromide staining. Under acidic conditions, AC₃₀ would be predicted to adopt an A-motif conformation as reported by Chakraborty et al. [19]. However, the addition of Ag⁺ to AC₃₀ at pH 3 did not cause retardation of the low mobility band. We envisage that Ag⁺ ions may insert into the existing A-motif without significant conformational changes to the DNA structure (Fig. S2, Lanes 1–2).

To investigate whether the platinum complex 1 could bind to the “on” state of the DNA logic gate (H⁺ = 1, Ag⁺ = 1), we performed parallel experiments in the presence of complex 1 or ethidium bromide (EB), a well-known DNA intercalator. Intriguingly, the band at pH 3 was retarded by complex 1 or EB (Fig. S2, Lanes 3–4), suggesting that complex 1 could bind to the A-motif conformation of AC₃₀, presumably via intercalation into double-strand DNA. Co-incubation of AC₃₀ with complex 1/EB and Ag⁺ ions at pH 3 also resulted in band retardation (Fig. S2, Lanes 5–6), indicating that complex 1 is able to effectively bind to the “on” state conformation (H⁺ = 1, Ag⁺ = 1) of the DNA logic gate. These results suggest that the enhanced luminescence response of the system to the analyte(s) is due to binding of the platinum(II) complex 1 to the DNA secondary structures induced by H⁺ and/or Ag⁺, presumably via an intercalative binding mode.

3.3. “OR” logic gate operation

For the operation of our logic gate, we defined the presence of H⁺ or Ag⁺ ions to correspond to an input signal of “1”. The absence of the ions would represent an input value of “0”. We first tested the response of 1–AC₃₀ to AgNPs under the optimized conditions using a titration experiment. In the absence of AgNPs under neutral conditions (H⁺ = 0, Ag⁺ = 0), the emission intensity of the platinum(II) complex 1 (10 μM) with AC₃₀ (15 μM) was weak due to the limited interaction between 1 and the single-stranded conformation of the oligonucleotide. The addition of AgNPs resulted in a concentration-dependent increase in the phosphorescence emission intensity of 1 at λₑₓ = 536 nm, with a relative phosphorescence enhancement (defined as I/I₀ where I₀ and I represent the
phosphorescence intensity of the system before and after the addition of analyte, respectively) of ca. 1.8 at [AgNP] = 40 nM (Fig. 5S). A detection limit of 20 nM for AgNPs was observed in the buffered system, with a linear detection range of 0–50 nM. This indicates that the presence of AgNPs only (H⁺ = 0, Ag⁺ = 1) is sufficient to trigger the "on" state of the 1-AC30 logic gate. The mechanism of emission enhancement is presumed to involve the Ag⁺ ion-stabilized formation of mismatched C-Ag(l)-C base pairs, allowing intercalation of 1 into the duplex structure resulting in strong phosphorescence.

We next investigated the effect of pH on the phosphorescence emission intensity of the 1-AC30 system, in the absence of AgNPs. At pH ≥ 6, the relative phosphorescence enhancement of 1 was very weak, presumably due to the single-stranded conformation of AC30. Lowering the pH to 5 resulted in a ca. 2.1-relative phosphorescence enhancement in the emission intensity of the metal complex (Fig. 2a). At pH 3, the emission enhancement was approximately 2.5-fold. This result is consistent with the formation of the proton-stabilized AH⁺−H⁻ ion pair by Chakraborty et al., who observed significant formation of a polyadenine duplex at pH 5.5 (but not pH 6.0) [19]. These results demonstrate that the DNA logic gate is sensitive to pH in the absence of AgNPs (H⁺ = 1, Ag⁺ = 0). In the presence of 100 nM of AgNPs, the probe was still pH dependent. A stronger phosphorescence emission was observed at pH ≤ 5 compared to pH ≥ 6, consistent with previous results (Fig. 2a). The highest emission is thus observed when both pH and AgNP inputs are present (H⁺ = 1, Ag⁺ = 1). This was also confirmed by an emission titration experiment with AgNPs performed at pH 3. A maximum fold-change response of approximately 2.9 was observed at [AgNP] = 100 nM (Fig. 3). By defining the cut-off for an "on" response to be a 50% increase in the emission intensity of the metal complex, this system functions as an OR logic gate for pH and AgNPs. The truth table for the operation of the OR gate is shown in Fig. 2b. The relative fold-change of the various combinations of inputs is summarized in Fig. 2c.

Cysteine has been reported to be a strong chelating agent for Ag⁺ and Hg²⁺ ions, and this has been utilized for the development of sensitive methods for the detection of cysteine [45,46]. Meanwhile, Chakraborty et al. have reported that the structure-switching event of polyA oligonucleotides could be controlled by the alternative addition of H⁺ or OH⁻ ions to the system [19], due to the destabilization of AH⁺−H⁻ A mismatched base pairs by hydroxide. Inspired by these phenomena, we reasoned that the addition of a suitable chemical reagent to remove one of the inputs could allow the relative contribution of the remaining input to be efficiently determined. The results showed that the addition of cysteine to AC30 in the presence of Ag⁺ ions decreased the luminescence of the system, presumably due to chelating or Ag⁺ ions by cysteine resulting in destabilization of the DNA duplex (Fig. 5Sa). Similarly, the addition of OH⁻ to the DNA logic gate removes H⁺ ions in solution, resulting in a decrease in luminescence intensity of the system (Fig. 5Sb). This allows the discrimination of the system response to particular input(s). Finally, addition of both cysteine and hydroxide ions reverts the DNA logic gate back into the "off" state (Fig. 5Sc). We envision the system could be potentially developed as a reusable label-free DNA logic gate system for H⁺ and Ag⁺ detection, thus reducing the cost of assay.

3.4. Selectivity of the strategy

We further demonstrated the selectivity of this system for Ag⁺ ions over other common heavy metal ions. We systematically
challenged the probe with 9 different interfering metal ions (Ba^{2+}, Na^+, K^+, Cr^{3+}, Cd^{2+}, Ni^{2+}, Li^+, Zn^{2+}, La^{3+}). Only slight increases in emission intensity were observed upon the addition of the interfering metal ions (Fig. S5). Only the addition of Ag^+ ions could result in a significant increase in the phosphorescence intensity, demonstrating the high selectivity of the system for silver ions.

### 3.5. AC\textsubscript{30} structure-switching response

We performed circular dichroism (CD) measurements to investigate the structure-switching response of AC\textsubscript{30} to Ag^+ ions (Fig. 4). The CD spectrum of AC\textsubscript{30} at pH 7.0 revealed a shoulder at 230 nm, a negative band at 260 nm and a positive band at 290 nm, which are characteristic of a single-stranded poly(AC) oligonucleotide [47] (Fig. 4a). The addition of Ag^+ ions resulted in a decrease of the positive signal at 285 nm, an inversion of the 220 nm peak from positive to negative, and the appearance of a new negative signal at 275 nm, which may indicate a Ag^+–induced structural transition of the oligonucleotide from a random coil structure to a C–Ag(I)–C mediated hairpin structure, characterized by the negative peaks at 220 and 275 nm [48,49]. By comparison, the CD spectrum of 1 μM AC\textsubscript{30} at pH 3.0, showed a positive band maximum at 290 nm, with a negative maximum at 260 nm and a slight positive peak at 225 nm, which are similar to the reported CD spectra of A\textsubscript{15} oligonucleotide at pH 3.0 [18] (Fig. 4b). These characteristic peaks indicate the formation of the A–H^+–H^+–A hairpin motif. Addition of Ag^+ ions decreased the intensity of the positive peaks at 225 nm and 290 nm, while the negative signal remained relatively unchanged, suggesting that the hairpin A-motif structure may have been perturbed by addition of Ag^+ ions. These results suggest that the addition of AgNPs induced structural changes in the conformation of the oligonucleotide, and that the precise secondary structure formed may also depend on the pH of the solution.

### 4. Conclusion

We have developed a label-free switch-on method for the detection of pH and AgNPs in aqueous solution using luminescent Pt(II) complex 1 as a probe. The operation of the probe is based on the stabilization of adenine–adenine or cytosine–cytosine mismatched base pairs by H^+ and Ag^+ ions, respectively. By utilizing a bifunctional adenine and cytosine-rich oligonucleotide AC\textsubscript{30}, the system could be developed into an OR DNA logic gate for pH and AgNPs. The addition of either input (pH ≤ 5, or AgNPs) is sufficient to trigger a luminescence enhancement of the metal complex, producing a switch-on output. The logic gate is highly selective for Ag^+ over a panel of other interfering metal ions tested. This “OR” logic gate system possesses several advantages, including simplicity, rapid response and cost-effectiveness. To our knowledge, the present study represents the first example of a luminescent DNA-based logic gate for pH and AgNPs, two putative indicators of pollution. This could be potentially developed as a sensory device for the simultaneous detection of H^+ and Ag^+ in-field, facilitating the rapid evaluation of water or soil quality without the need for expensive sensing devices. Furthermore, we have demonstrated that the relative contributions of the logic gate inputs could be determined by the addition of suitable “masking agents” such as OH⁻ and cysteine. We anticipate that our work may provide a basis for the future development of DNA logic gates could potentially form the basis of “smart” analytical detection technologies that give single distinct output depending on the concentration of various metal ions or pH inputs.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.aca.2012.04.041](http://dx.doi.org/10.1016/j.aca.2012.04.041).

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