Development of a highly sensitive label-free DNA based fluorescent sensor for cisplatin detection

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Abstract. Cisplatin, or cis-diamminedichloroplatinum (II), is a platinum-based chemotherapeutic drug. It is widely used against many types of cancer. However, there are various side effects in patients taking cisplatin. The side effects are also found in chemotherapists or health workers exposed to contaminating cisplatin in workplace. Therefore, there is a need for sensors for cisplatin contamination detection. This work reports the development of a highly sensitive fluorescent sensor using single stranded ssDNA as a sensing element. It contains consecutive guanine bases to allow an efficient cisplatin binding. Then, an enzyme-free DNA amplification technique called catalyzed hairpin assembly (CHA) was employed to increase the sensor sensitivity. It comprises two hairpin DNAs (H1 and H2) which are in meta-stable states and can form a hybrid only in the presence of the cisplatin sensing ssDNA described above. H1 is designed such that after the hybrid formation, a newly exposed overhang forms a G-quadruplex (GQ). The GQ formation can be monitored by mixing a GQ binding dye called Thioflavin T (ThT) in the solution. ThT fluorescence quantum yield is negligible if ThT is free in solution but increases over 1000 folds when bound to GQ. The formation of H1:H2 hybrid and ThT-GQ complex is disrupted if cisplatin binds to the sensing ssDNA. Therefore, the decrease in ThT fluorescence signifies the increase in cisplatin concentration and vice versa. The developed sensor capable to detect the cisplatin in the range of 500 – 7500 nM and the limit of detection is 182 nM. The merit of this novel sensor is that it is label-free therefore economical yet highly sensitive. It is expected that the sensor can help monitor cisplatin contamination in chemotherapeutic workplaces.

1. Introduction
Cisplatin, or cis-diamminedichloroplatinum (II), is a chemotherapeutic drug in platinum group [1]. The mechanism of action involves the bonding between a platinum atom from cisplatin and two nitrogen atoms from two purine bases [2]. This platinum-DNA adduct can be either an intrastrand crosslink or an interstrand crosslink [2]. It is widely used against many types of cancer, such as germ cell tumors, lymphomas, skin cancer and sarcomas [3]. However, there are various side effects in patients taking
cisplatin, such as toxicity to liver and kidney and vomiting [4]. The side effect also occurs in chemotherapists or health workers exposed to contaminating cisplatin in workplace at high level [5]. Thus, it is important to detect the contamination of cisplatin in, e.g., chemotherapy units.

Currently, there are several methods for cisplatin detection, including high-performance liquid chromatography (HPLC) with a limit of quantitation (LOQ) as low as 6.6 μM (2 μg/ml) [6], mass spectrometry (MS) with a limit of detection (LOD) of 2.3 nM (0.7 ng/ml) [7] and inductively coupled plasma mass spectrometry (ICP-MS) with an LOD of 3.3 nM (1 ng/ml) [8]. Even though they offer usable detection limits, the techniques are time consuming, complicated and expensive. Chemical sensors and biosensors, on the other hand, are usually fast, simple and low cost [9].

In this work, a simple yet sensitive cisplatin biosensor is constructed based on a DNA nanotechnology. The sensing mechanism is based on a strong binding between cisplatin and dual guanine bases in the sensing DNA [2]. A non-enzymatic amplification method called catalyzed hairpin assembly (CHA) method [10] is used in conjunction with a fluorescent probe to ensure high sensitivity. However, unlike most fluorescent sensors, this work opts for a label-free method to minimize the cost.

2. Materials and Methods

2.1. Sensing strategy

The sensor mechanism can be separated into two parts: the cisplatin binding with a sensing single stranded DNA (ssDNA) (figure 1a) and the signal amplification via CHA (figure 1b). The sensing ssDNA plays a double role of either binding cisplatin or catalyzing the CHA reaction. In the absence of cisplatin, ssDNA catalyzed the CHA reaction by opening the DNA hairpin H1, which in turn opens the DNA hairpin H2 (figure 1b). After the formation of H1:H2 hybrid, ssDNA is freed and ready for the next round of catalysis. This continues until the hairpin H1 and H2 are exhausted from the solution. However, in the presence of cisplatin, the formation of cisplatin-ssDNA adduct (figure 1a) virtually stops all the downstream CHA reactions. Therefore, a trace level of cisplatin can be easily measured indirectly (but easily) from the inhibition of the H1:H2 hybrid formation. To report the creation of H1:H2 hybrid, we placed a G-quadruplex (GQ) forming domain at the 3’ end of H1 (Table 1, underlined). This domain is hidden in the stem when H1 is in the hairpin form but becomes an overhang and forms a GQ when H1 hybridizes with H2. Then a GQ specific fluorescent dye called Thioflavin T (ThT) is mixed in the solution to bind with the GQ. ThT emits virtually no fluorescence in the free form but becomes extremely bright when bound with a GQ [9]. Therefore, high ThT fluorescence signifies low cisplatin concentration and vice versa.

![Figure 1. The sensing mechanism comprising two parts: (A) Cisplatin binding and (B) Signal amplification.](image)
2.2. Chemicals and apparatus
All oligonucleotides were from Macrogen (Seoul, Republic of Korea) with sequences shown in Table 1. The underlined portion of H1 is the G-quadruplex (GQ) forming domain. Cisplatin and the rest of the chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

Table 1. Oligonucleotides used in this study.

| Name     | Length (base) | Sequence (from 5’ to 3’ end)                  |
|----------|---------------|-----------------------------------------------|
| H1       | 76            | ATCCTACATCCACCCTAAGCCCTAATGGATGGATGGATGGATGGGCTAGGGCTAGGGCTAGGG |
| H2       | 51            | TGCAACCATCCTACATCCATTAGGGCTAGGGCTAGGGTGGATGGAT |
| ssDNA    | 32            | TTAGGGCTAGGGCTAGGGCTAGGGATGGATGGAT |

Remark: the underlined section on H1 denotes a G-quadruplex forming domain.

Fluorescence measurements were performed using a fluorescence spectrometer (LS-55, Perkin Elmer, USA) at room temperature.

2.3. Sensor preparation and fluorescence measurement
H1 and H2 in 100 mM sodium phosphate buffer, pH 5.0 was heated to 90°C for 10 min and slowly cooled down to room temperature for 1 h before use. Cisplatin preparation was done by adding AgNO3 and incubated for 15 h before use. ssDNA, ThT and cisplatin were diluted in potassium phosphate buffer (500 mM, pH 5.0). The cisplatin titration was performed by mixing cisplatin at a desired concentration with ssDNA (200 nM) in 500 μl centrifuge tube and incubated at room temperature for 2 h. Then H1, H2 (200 nM each) and ThT (500 nM) were added and incubated at room temperature for 2 h. The volume of reaction was fixed at 150 μl. The fluorescence response (F) was obtained by integrating the ThT emission spectrum from 440 – 700 nm (10 nm slit width) at 425 nm excitation (5 nm slit width). The sensor response if shown as the ratio between F0 and F (or F0/F) where F0 is the integrated fluorescence intensity in the absence cisplatin.

3. Results and Discussion
The fluorescence emission from the sensor decreased significantly as the concentration of cisplatin increased from 0 to 10,000 nM as shown in figure 2A. The calibration graph in figure 2B obtained from the raw signal shows a linear range of 500 – 7,500 nM (inset) with the limit of detection (LOD) of 182 nM and the limit of quantification (LOQ) of 608 nM. The LOD and LOQ were calculated from 3Sa/b and 10Sa/b, respectively, where Sa and b are deviation of the intercept and the slope of the calibration graph [11].

Figure 2. (A) The sensor’s fluorescence emission spectra at various concentrations of cisplatin from 0 to 10,000 nM. (B) A calibration graph with the inset showing the linear range.
4. Conclusion
We successfully developed a highly sensitive yet low cost sensor for cisplatin detection based on DNA equipped with a non-enzymatic amplification unit and a label-free fluorescence signal generation. The catalyzed hairpin assembly (CHA) reaction used as a signal amplification improves the limit of detection almost 4 times compared with a previous work [12]. The sensor is able to detect cisplatin in the range of 500 – 7500 nM, which is enough to detect cisplatin contamination in workplaces.

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