Label-Free Liquid Crystal Biosensor Based on Specific Oligonucleotide Probes for Heavy Metal Ions

Shengyuan Yang,†‡ Chao Wu,† Hui Tan,† Yan Wu,† Shuzhen Liao,† Zhaoyang Wu,*,† Guoli Shen,† and Ruqin Yu†

†State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China
‡College of Public Health, University of South China, Hengyang 421001, P. R. China

Supporting Information

ABSTRACT: In this study, to enhance the capability of metal ions disturbing the orientation of liquid crystals (LCs), we designed a new label-free LC biosensor for the highly selective and sensitive detection of heavy metal ions. This strategy makes use of the target-induced DNA conformational change to enhance the disruption of target molecules for the orientation of LC leading to an amplified optical signal. The Hg²⁺ ion, which possesses a unique property to bind specifically to two DNA thymine (T) bases, is used as a model heavy metal ion. In the presence of Hg²⁺, the specific oligonucleotide probes form a conformational reorganization of the oligonucleotide probes from hairpin structure to duplex-like complexes. The duplex-like complexes are then bound on the triethoxysilylbutyraldehyde/N,N,N-dimethyl-N-octadecyl (3-aminopropyl) trimethoxysilyl chloride (TEA/DMOAP)-coated substrate modified with capture probes, which can greatly distort the orientational profile of LC, making the optical image of LC cell birefringent as a result. The optical signal of LC sensor has a visible change at the Hg²⁺ concentration of low to 0.1 nM, showing good detection sensitivity. The cost-effective LC sensing method can translate the concentration signal of heavy metal ions in solution into the presence of DNA duplexes and is expected to be a sensitive detection platform for heavy metal ions and other small molecule monitors.

Liquid crystals (LCs) are materials typically composed of rod-like molecules, which possess short-range positional but long-range orientational order,1 and can be used to amplify and transduce the surface binding events into optical outputs that can be easily observed.2−19 These idiosyncrasies, combined with their speed of response to an external stimulus, make them well-suited as “sensing elements”. Recently, LC-based sensors have attracted particular attention for their unique characters. Compared to traditional analytical approaches, the LC-based sensors permit label-free detection with high sensitivity and without the requirement of complex instruments and even the need of electrical power, making them sufficiently simple and well suited for the primary screening assay of analytes performed away from central laboratories.2,3 Several studies have reported that LC-based sensors for the detection of DNA hybridization,3−7 protein binding event,8−10 enzymatic reaction,11−13 enzyme inhibitors,14,15 surface-active reagents,16,17 antigen–antibody,18 and so on. Most of these LC-based detections rely on the biological macromolecule binding events which can change the anchoring behaviors of LCs on the surface, but as yet, few LC-based sensors have been proposed for the detection of heavy metals ions since the metal ions of low concentration are not quite effective to disrupt the orientation of LC molecules due to their small intermolecular interaction. Hu et al.19 have reported that only more than micromoles level of metal ions can result in observable optical signal responses. On the other hand, many metal ions are very similar and sometimes even identical in charge, ionic radius, and other specialties, making them very difficult to detect at ultralow concentrations with no interference from other metal ions. In view of the superior properties of LC-based sensors and

Received: October 19, 2012
Accepted: December 7, 2012
Published: December 7, 2012
the importance of heavy metal ion detection, it is interesting and significant to find a new way to design a highly sensitive and selective LC sensor for heavy metals ions.

Our previous work has demonstrated that the high density of binding ds-DNA can obviously disrupt the orientation of LC and produce an obvious response in the optical textures. Therefore, we consider that it is also possible to develop highly sensitive LC sensors for metal ion assays by translating the concentration information of metal ion to the binding event of DNA. In fact, most functional DNA requires metal ion cofactors for their structure or function, and many functional DNAs show high metal-binding affinity and specificity. Herein, we try to design a new type of LC-based biosensor for the detection of heavy metals using functional DNA with specific recognition. Due to the analysis significance of Hg$^{2+}$ ions as a highly toxic environmental contaminant and its unique property to bind specifically to T−T base pairs in DNA duplexes, it was chosen as a representative model of heavy metal ions to construct a LC-based biosensor.

Although the T−Hg$^{2+}$−T-based detection systems have been applied in fluorescent biosensors, colorimetric biosensors, and electrochemical sensors, this work is the first demonstration using the target dominated DNA hybridization to produce the enhanced optical signal in the field of LC biosensors. In this LC sensing system, the T−Hg$^{2+}$−T interaction can form stable DNA duplexes, which will sensitively distort the orientational profile of LCs, leading to an amplified optical signal and thus can achieve the sensitive LC sensing detection of small molecules. This label-free LC-based imaging system is simple and cost-effective, and it is anticipated to be a powerful tool to assess the heavy metal ions contamination.

The mechanism of the LC biosensor for the detection of Hg$^{2+}$ ion is shown in Scheme 1. It is composed of three elements: an 18-mer oligonucleotide with an amino at its 5′ end as a capture probe (probe A), a hairpin oligonucleotide (probe B) which is known to have a high specificity over these linear chain probes, and an appropriate oligonucleotide (probe C) partially complementary with the probe B loop sequence except for five T−T mismatches. The capture probe was first immobilized on the plane glass slide surface, which was chemically functionalized with a self-assembling triethoxysilyl-butyraldehyde/N,N-dimethyl-N-octadecyl (3-aminopropyl) trimethoxysilyl chloride (TEA/DMOAP) film. The TEA provides aldehyde groups for coupling the terminal amino groups of probe A, and the DMOAP orientates LCs perpendicularly to provide a uniform dark background. The shorter probes are preferable to the longer probes for more efficient surface immobilization and surface hybridization due to the significantly decreased steric hindrance, and the length of oligonucleotide has an effect on the orientation of LCs. Therefore, an 18-mer oligonucleotide is selected as a capture probe to ensure a good signal-to-noise ratio and sufficient complementary base pairs for the stable hybridization. The loop structure of probe B contains five T-bases for Hg$^{2+}$ binding, and the 3′-terminal is partially complementary with the capture probe. When the sensing interface is incubated with the solutions containing Hg$^{2+}$ and the probes B and C at room temperature, the probe B hybridizes with the probe C via the specific T−Hg$^{2+}$−T interaction and its conformation is changed from the stem-loop structure to the formation of rigid rod-like duplex DNA; subsequently, the 3′-terminal of the probe B hybridizes with the capture probe. The bound DNA duplexes on the LC sensor substrate surface can greatly change the surface topology and further induce a homeotropic-to-tiled transition of the LC molecules surrounding them. Due to the long-range order inherent in LC phases, the homeotropic-to-tiled transition of the LC molecules will cause a distorted orientational profile inside the LC cell, making the optical image of LC cell birefringent as a result. In the absence of Hg$^{2+}$, the hairpin probe can not hybridize with the probe C because the melting temperature (T_m) of DNA duplex with the T−T mismatches is lower than the incubating temperature. Accordingly, almost no ds-DNAs are bound to the LC sensor substrate surface, the long alkyl chain layer of DMOAP still predominated in inducing the homeotropic alignment of LC molecules, and the optical image is uniformly dark.

Moreover, one can precisely modulate the T_m of two probes by controlling the number of T−T mismatches in the sensing system. The DINAMelt web server was used to predict the thermodynamical stability of the DNA duplex of the probe B and the appropriate oligonucleotide probes (probe C1−5) which are complementary to the probe B loop sequence except for various numbers of T−T mismatches. According to
the estimation of the DINAMelt web, the $T_m$ of the probes B and C1−5 are decreased from 43.6, 31.7, 28.1, and 19.0 °C with an increasing number of T−T mismatches. It can be found that the $T_m$ of the DNA duplex can be well controlled below
the operating ambient temperature (25 °C) when the probe C contains five T–T mismatches. As a proof-of-concept experiment, an aqueous solution of probes A, B, and C1 was selected for a prototype sensor system for Hg2+ at room temperature.

The optical signal characteristics of the LC sensor for different concentrations of Hg2+ are shown in Figure 1. It can be observed that the LC-based method has a good signal-to-background contrast and a clear distinction between positive and negative results. The optical image is uniformly dark in the absence of Hg2+, showing a zero-background for the sensing system, and some distinct bright spots appear on the optical image with the addition of Hg2+ up to 0.1 nM, which can be easily distinguished from the dark background, and the number of bright spots and birefringent textures in the optical images increases with the Hg2+ concentration from 0.1 to 5.0 nM. This result suggests that the binding of Hg2+-mediated DNA duplexes on the LC substrate surface leads to the change of LC orientation and the introduction of Hg2+ is a negligible factor on the change of the optical signal of LC sensor.

To further characterize the structural rearrangement of the hairpin DNA probe B to the rod-like DNA double-stranded helix in the presence of Hg2+ and probe C, some fluorescence experiments were performed. It is known that the DNA hybridization can be easily monitored with intercalation dyes, such as SYBR Green I (SG), which shown high selectivity and sensitivity toward ds-DNA due to its different nature upon interaction with ss-DNA and ds-DNA. The fluorescence spectra (see Figure S-1 in the Supporting Information) show that, in absence of Hg2+ ions, the weak fluorescence intensity is observed since the probe C in solution exists in a random-coil structure and the probe B only has seven base pairs in the stem part. After addition of different concentrations of Hg2+, an obvious change of the fluorescence intensity appears as shown in curves b and c. The intensity of fluorescence emission at 533 nm distinctly increases, and the maximum emission wavelength gradually blue-shifted to 530 nm. This phenomenon is related to the change of oligonucleotide conformation, and it implies that the probe B successfully hybridized with probe C and more ds-DNAs appeared in solution in the presence of Hg2+.

The atomic force microscope (AFM) was applied to characterize the exterior state of the substrates before and after addition of Hg2+ ions (shown in Figure 2). Figure 2A is the AFM image of the TEA/DMOAP-coated substrate modified with capture probe. The dark brown level corresponds to the minimum of the height range and the white level to the maximum height.22 The root-mean-square (rms) roughness (Rq) is 0.591 nm, and the largest peak to valley height (LPVH) is 8.74 nm. Figure 2B shows a region of the capture probe modified substrate after addition of the probes B and C in the absence of Hg2+ ions. The Rq is 0.410 nm, and the LPVH is 6.55 nm. There is no appreciable distinction in the exterior structure between Figure 2A&B, in which white dots are globular shape, and their density is almost consistent. The surface morphology of capture probe modified substrate after incubation with the solution containing probes B and C and Hg2+ ion is shown in Figure 2C. There are apparent differences in the exterior structure between Figure 2A&C. The Rq and LPVH values of Figure 2C significantly increase to 2.05 and 27.5 nm, respectively, and the shapes appear rod-like. The Hansma group22 has proved that, under AFM, short ds-DNA molecules are generally rod-like in shape, while ss-DNA molecules are globular. That is to say, in the absence of Hg2+ ion, few ds-DNA are bound to LC substrate surface and the surface topographical structure has no visible change. In contrast, the probe B can well hybridize with probe C via the stable specific T–Hg2+–T interaction in the presence of Hg2+, and a large number of ds-DNAs are bound to the substrate surface, greatly changing the surface topology.

The selectivity of the method was investigated by substituting Hg2+ (0.1 nM) in the hybridization buffer with various metal ions such as K+, Zn2+, Mg2+, Ca2+, Pb2+, Cd2+, Mn2+, Cu2+, Al3+, and Fe3+ and a mixture of ions (1 μM each). As displayed in Figure S-3 in the Supporting Information, the birefringent textures in the optical images are only observed in the presence of Hg2+, while there is almost no signal response for other metal ions. This observation denotes that the LC sensing detection has an impressive specificity for Hg2+.

To evaluate the capability of the LC biosensor for the detection of Hg2+ in environmental water samples, two samples from river water were tested. The result was shown in Figure S-4 in the Supporting Information. It can be found that obvious birefringent textures appears in the optical images when the sensor interface is incubated with river water samples, and the optical image of the sample with 5 nM Hg2+ shows more obvious birefringent textures than that of the sample with 1 nM. This result well demonstrates the potential of the developed LC sensor for practical application in Hg2+ monitoring, especially in primary screening assays of Hg2+.

In summary, we proposed a new type of LC-based biosensing strategy for heavy metal ions. This strategy translating the small molecule detection into the DNA hybridization behavior leading to an amplified optical signal can well enhance the capability of metal ions disturbing the orientation of LCs and achieve the sensitive LC sensing detection of heavy metal ions. With amplification by DNA hybridization behavior, the sensitivity for the detection of Hg2+ can achieve 0.1 nM. The proposed sensor is simple and label-free, does not require costly equipment, and is well suited for the primary screening assay of analytes performed away from central laboratories. Although there have been some reports on the heavy metal ion detection in other fields, this is the first demonstration using target dominated DNA hybridization to produce enhanced optical signal in the field of LC biosensors. This LC biosensing strategy is a promising tool for the study of other toxic metallic ions and small molecules.

ASSOCIATED CONTENT

Supporting Information
Experimental details and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*E-mail: zywu@hnu.edu.cn. Tel./Fax: +86-731-88821989.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 21175037, 21277042, and 11205085) and New Century Excellent Talents in University (NCET-11-0132).
REFERENCES


