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Label-free liquid crystal biosensor for L-histidine: A DNAzyme-based platform for small molecule assay

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ABSTRACT

We have developed a novel DNAzyme-based liquid crystal (LC) biosensor with high sensitivity for L-histidine, which is based on L-histidine-mediated formation of DNA duplexes by cleaving DNAzyme using L-histidine, resulting in a remarkable optical signal. Firstly, an optimal amount of capture probe is bound to the glass slide, which changes the surface topology as little as possible and shows a zero-background for the sensing system. When the DNAzyme molecule is cleaved by the target, L-histidine, a partial substrate strand is produced, which in turn can hybridize with the capture probe, forming a DNA duplex. The DNA duplexes induce LC molecules to undergo a homeotropic-to-tiled transition, obtaining a remarkable optical signal. The results show that the DNAzyme-based LC biosensor is highly sensitive to L-histidine with a detection limit of 50 nM. Compared with previously reported multi-step amplified methods, this newly designed assay system for L-histidine has no amplified procedures with comparable sensitivity. This method is an unprecedented example of DNAzyme-based LC biosensor for small molecules, which has potential to offer a DNAzyme-based LC model used in various targets.

1. Introduction

DNAzymes are DNA molecules obtained by in vitro selection, which possess catalytic activity (Shoji et al., 2007; Joyce, 2007). However, most DNAzymes need certain neutral molecules or metal ions as cofactors for the purpose of expanding their catalytic functionalities (Freisinger and Sigel, 2007; Sigel and Pyle, 2006). Since the discovery of the first DNAzyme (Breaker and Joy, 1994), researchers have attempted to convert DNAzymes into novel biosensors for using in the detection of metal ions such as Cu(II) (Carmi et al., 1996; Cuenoud and Szostak, 1995), Pb(II) (Breaker and Joy, 1994; Pan and Uhlenbeck, 1992) and Zn(II) (Santoro et al., 2000; Li et al., 2000) or neutral molecules such as L-histidine (Liang et al., 2011; Kong et al., 2011). The signal transduction mechanisms of these biosensors are usually based on fluorescence, colorimetry and electrochemistry. The DNAzyme-based sensors have a high selectivity, even in the complex environmental system. However, almost all of these sensors have to label the DNAzyme with signal molecules to realize the production of an efficient signal output, which need complicated synthetic steps. In addition to DNAzyme modifications, the other important problem is that some signal amplified procedure is needed to improve sensitivity in order to apply in complex environmental sufferring background interference.

Liquid crystals (LCs) are materials, which possess short-range positional but long-range orientational order (Adgate et al., 2009). The orientation of LCs is sensitive to the change of a bounding interface. This notable characteristic makes LCs as ideal candidates for developing powerful and advanced sensing systems, such as label-free with high sensitivity but no requiring complex instruments. LCs has also been used in transform biomolecular or chemical binding events into amplified visible optical signals (Gupta et al., 1998; Kim et al., 2000; Bi et al., 2009; Tan et al., 2010; Price and Schwartz, 2008; Liao et al., 2011). Owing to the superior properties of LCs, researchers have paid particular attention to develop LC-based sensors. However, most of those sensors are applied in the biological macromolecule binding events, such as protein–protein, protein–ligand and nucleic acid hybridization events (Gupta et al., 1998; Kim et al., 2000; Gupta and Abbott, 1997; Xue and Yang, 2007; Hartono et al., 2008). The optical signal of LC-based sensors is thought to in proportion to the size and amount of macro-biomolecules causing obvious topographic changes of the well-defined glass slides, and then disturbing the orientation of LC. So far, few examples of LC-based sensors have been used in small molecules.

Therefore, it is interesting and significant to design a novel DNAzyme-based LC biosensor for small molecules, which can avoid the complicated DNAzyme modifications and amplified procedure. L-histidine, a small molecule is chosen as a model. In this system, the bound capture probe changes the surface topology

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of glass slides for the LC sensor as little as possible, displaying a low-background for this system. On the other hand, the unmodified DNAzyme is cleaved by L-histidine to release a partial substrate sequence. The partial substrate sequence hybridizes with the capture probe, and then DNA duplexes are formed on the surface of glass slide. The DNA duplexes can obviously change the glass slide surface topology, which further induce an orientational transition of the LCs, and then resulting in an amplified optical signal. To our best knowledge, this is an unprecedented DNAzyme-based LC biosensing system used to detect the target. In comparison with previously reported DNAzyme-based sensors, this method has no complicated DNAzyme modifications and amplified procedures with comparable sensitivity. This revolutionized strategy would open up a new LC-based approach for the study of small molecules.

2. Material and methods

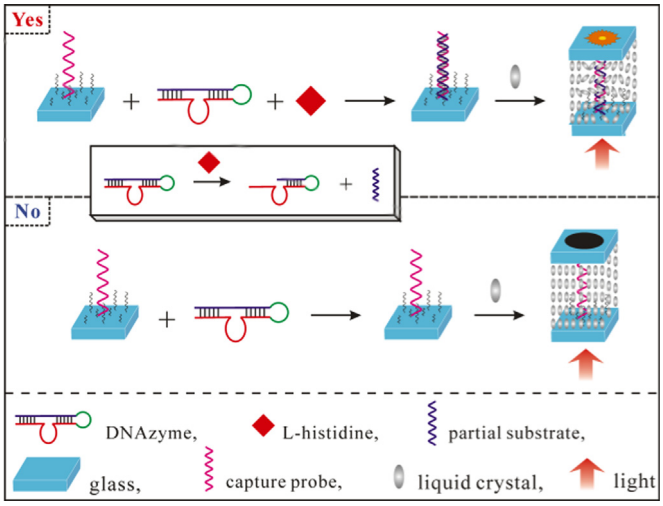
2.1. Chemicals and materials

L-histidine, phenylalanine, arginine, lysine, N, N-dimethyl-n-octadecyl (3-aminopropyl) trimethoxy-silyl chloride (DMOAP), sodium dodecyl sulfate (SDS) and $NaBH_3CN$ were purchased from Sigma-Aldrich. Nematic LC 4-cyano-4′-pentylbiphenyl (5CB) was obtained from Heibei Huajing Scientific and Technological Development Co., Ltd. (Heibei, China). Triethoxysilybutyraldehyde (TEA) was purchased from Hong Kong Advanced Technology & Industrial Co., Ltd. Mylar (~23 μm, polyimide membrane) was purchased from Guangzhou Shu Ge Electric Co., Ltd. Other reagents were analytical purity. Ultrapure water was used throughout all experiments. All oligonucleotides used in the work were obtained from TaKaRa Biotechnology Co., Ltd. (Dalian, China) and purified by High-Performance Liquid Chromatography. Thermodynamic parameters and secondary structures of all oligonucleotides were calculated using bioinformatics software (http://mfold.rna.albany.edu). The sequences of the synthesized oligonucleotides are given in Table 1. Probe 1 was designed as a capture probe. Probe 2 was designed as a DNAzyme oligonucleotide. DNAzyme sequences contain three main components: the sequence of AGATCATGTGACGGACATrAGGAAGAGATG is designed as a substrate (corresponding to blue portion in Scheme 1), the sequence of TTTTTTTTTT is designed as a polyT sequence (corresponding to green portion in Scheme 1) and the sequence of CATCTCTTAACGGGGCTGTGCGGCTAGGAAGTAATGTCCTCC is designed as an enzyme sequence (corresponding to orange portion in Scheme 1). The two underlined parts of the probes are complementary. The rA in probe 2 is the site cleaved by L-histidine.

Premium grade glass slides were obtained from Xinhua Laboratory Glassware Company (Haimen, China).

2.2. Glass slides pretreatment and decoration (Liao et al., 2011)

The glass slides were cleaned with freshly prepared piranha solution (70% H_2SO_4, 30% H_2O_2) at 80 °C for 1 h to remove all organic contaminants. *Caution: piranha solution reacts violently with organic materials and should be handled with extreme caution; do not store the solution in closed containers!* The glass slides were then rinsed with copious amounts of ultrapure water, dried under a stream of nitrogen, and stored in a 120 °C vacuum oven for at least 3 h before using.



Scheme 1. Description of the high sensitive DNAzyme-based LC biosensor for L-histidine. Only L-histidine can cleave the DNAzyme and release partial substrate, which hybridizes with the capture probe forming ds-DNA on the glass slide. The ds-DNA induces a homeotripic-to-tiled transition of the LCs, resulting in a birefringent texture of the optical image for LC cell.

Some cleaned glass slides were immersed in an aqueous solution containing 0.5% (v/v) DMOAP at RT for 10 min and then rinsed with copious amounts of ultrapure water. The DMOAP-coated glass slides were dried under a stream of nitrogen and heated in a 110 °C vacuum oven for 1 h.

Some cleaned glass slides were immersed in ethanol solution containing 1% (v/v) TEA and 0.5% (v/v) DMOAP at 80 °C for 1 h, rinsed with ethanol to remove any physically absorbed TEA/DMOAP and then ultrapure water, dried with nitrogen flow, and then stored at 110 °C for 1 h.

2.3. Probe 1 immobilization

Capture probe (probe 1) employed in this work was first dissolved in a 20 mM Tris–HCl buffer solution (pH 8.0) containing 100 mM Mg^{2+} and 10 mM $NaBH_3CN$. 100 μL solution of capture probe (100 nM, probe 1) was dropped to the TEA/DMOAP-decorated glass slides, then incubation at 37 °C for 2 h, rinsed with a solution of 0.3 M sodium chloride / 0.03 M trisodium citrate (2 × SSC, pH 7.0) containing 0.01% (v/v) SDS and ultrapure water to eliminate the nonspecific-bonded capture probe. The excess aldehyde group in the glass surface was blocked by using 100 mM glycine at RT for 1 h.

2.4. The DNAzyme-based LC biosensor for L-histidine

Firstly, 100 μL mixture solutions contain different concentrations of L-histidine and 100 nM DNAzymes (probe 2, in 10 mM Tris–HCl buffer solution (pH 7.4) containing 150 mM Na^+, 5 mM Mg^{2+}) were dropped on the glass slide immobilized with probe 1 at room temperature for 1 h. The unbounded DNA probes were rinsed with 2 × SSC containing 0.01% (v/v) SDS and abundant ultrapure water. Subsequently, the LC cells were fabricated by spacing a DMOAP-coated glass and a TEA/DMOAP-coated glass with two thin strips of Mylar and then held together with small binder

Table 1
Synthesized oligonucleotides (5′-3′) used in the experiments.

Probe 1 (capture probe)	ATGTCCTCCGTCACATGATCTTTTTT-$(CH_2)_6$-NH_2
Probe 2 (DNAzyme)	AGATCATGTGACGGACATrAGGAAGAGATGTTTTTTTTTTCATCTCTTAACGGGGCTGTGCGGCTAGGAAGTAATGTCCTCC

clips. Finally, the optical appearance of the samples was examined using a polarized light microscope in transmission mode under crossed polarizer. The images were captured by a digital camera mounted on the microscope.

2.5. Fluorescent spectra to investigate the viability of our strategy

Fluorescent spectra were monitored by the F-7000 (Hitachi, Japan) to investigate target-induced fluorescence changes in the presence and absence of L-histidine. The DNAzyme (200 nM, in 10 mM Tris–HCl buffer solution (pH 7.4) containing 150 mM Na^+, 5 mM Mg^{2+}) was directly incubated with SYBR Green I (SG) for 30 min at room temperature, after which the fluorescent spectrum was recorded. The DNAzyme (200 nM) was firstly incubated with L-histidine (1000 μM) for 20 min at room temperature. After the addition of SG, the mixture was incubated for another 30 min at room temperature, after which the fluorescent spectrum was recorded.

2.6. Gel electrophoresis to investigate the viability of our strategy

The mixture solutions contain 1 μM capture probe, 1 μM DNAzyme, and 5 μM L-histidine. After incubation at room temperature for 1 h, the resultant mixture was analyzed using gel electrophoresis in 3% (w/w) polyacrylamide containing 1% (w/w) ethidium bromide. The gel was visualized using a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company).

3. Results and discussion

3.1. Principle of the DNAzyme-based LC biosensor for L-histidine

The principle of the DNAzyme-based LC biosensor used for L-histidine is depicted in Scheme 1. The detection scheme is composed of two main parts: one is the unmodified DNAzyme (probe 2), a molecular for recognizing L-histidine (see Table 1), the other is the capture probe (probe 1) with amino at its end, as a complementary oligonucleotide to the DNAzyme product cleaved by L-histidine. The DNAzyme includes three main components, namely, substrate (blue portion), enzyme sequence (orange portion) and polyT sequence (green portion). In this system, the surface of the glass slide was firstly functionalized with a self-assembling triethoxysilylbutyraldehyde / N, N-dimethyl-N-octadecyl (3-aminopropyl) trimethoxysilyl chloride (TEA/DMOAP) film, and then the probe 1 was immobilized on the glass slide surface by coupling aldehyde groups of TEA.

3.2. The feasibility STUDY of the principle

The Abbott group has reported that the orientational changes of LCs are closely relevant to the changes of topographical structure of the surface close to LCs (Skaife and Abbott, 2001). In this DNAzyme-based LC sensor system, the different modification of the glass slide surface result in the different degree of LCs orientational changes and produce a distinguishable response in the optical textures. Due to the long alkyl chain layer of DMOAP orientates LCs perpendicularly. Firstly, the TEA / DMOAP mixed film can effectively induce homeotropic alignment of LCs, making a uniform dark background optical image as a result (Fig. 1a). In the absence of L-histidine, the capture probe immobilized on the glass slide surface cannot hybridize with the uncleaved partial substrate of DNAzyme due to the strong intramolecular hybridization of the DNAzyme molecule. Accordingly, almost no ds-DNAs are formed on the glass slide surfaces, as shown in Fig. 1b, there are a few bright spots when the capture probe is immersed into the solution containing DNAzyme but no L-histidine. However, when the glass slide immobilized with capture probe is incubated with the solutions containing both L-histidine and the DNAzyme at room temperature, the DNAzyme is cleaved by L-histidine and release partial substrate for the hybridization with the capture probe forming ds-DNA. The bound ds-DNAs on the glass slide surface can obviously change the surface topology and further induce LCs to undergo a homeotropic-to-tiled transition. Owing to the long-range order inherent of LCs, this transition of the LCs will result in a distorted orientational profile, making birefringent texture of the optical image for LC cell as shown in Fig. 1c. In order to prove our assumption that the homeotropic-to-tiled transition of the LC molecule is due to the formation of ds-DNA on the glass slide surface, some control experiments are carried out, such as (1) in the presence of DNAzyme and/or L-histidine but without the probe immobilization on the surface, and (2) in the presence of only L-histidine or DNAzyme with the probe immobilization on the surface. The experimental results indicate that the optical images of the control experiment are same as a uniform dark background appearance (Fig. S1), which accord with our assumption.

To further characterize the structural change of DNAzyme in the presence and absence of L-histidine, some fluorescence experiments were carried out. SYBR Green I (SG), an intercalation dye was selected for monitoring the DNA hybridization, which can distinguish ss-DNA from ds-DNA with high sensitivity and selectivity as its nature interaction. The fluorescence spectrum shows strong intensity in the absence of L-histidine (curve a in Fig. 2). This is because that DNAzyme in solution presents in a ds-DNA structure, which can be intercalated by SG easily. After the addition of L-histidine, the weak fluorescence intensity is shown in

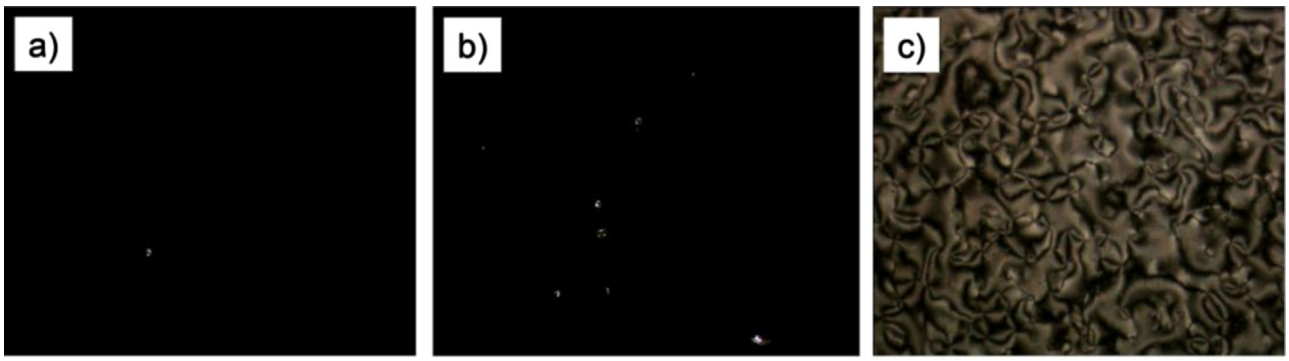


Fig. 1. Optical images under cross polarizer of LC cells with nematic LC 4-cyano-4′-pentylbiphenyl (5CB) in different conditions: (a) self-assembled TEA/DMOAP film, (b) immobilization of capture probe (100 nM) after immersing into the solution of DNAzyme (100 nM), (c) immobilization of capture probe (100 nM) after immersing into the solution of DNAzyme (100 nM) and L-histidine (500 nM).

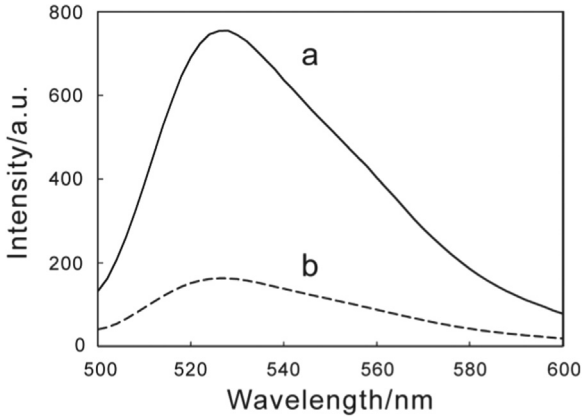


Fig. 2. Fluorescence responses of the sensing system under different conditions: (a) 200 nM DNAzymes; (b) 1000 μM L-histidine and 200 nM DNAzymes.

curve b. This phenomenon is due to the change of DNAzyme conformation from ds-stranded to ss-stranded DNA in the presence of L-histidine.

Gel electrophoresis was also performed, which not only indicated the change of DNAzyme, but also implied the formation of DNA duplexes in the presence of L-histidine. As shown in the Fig. S2, there were two bright bands when adding L-histidine into the mixture solution containing capture probe and DNAzyme (Fig. S2, lane 4), which is different from those containing only capture probe containing 26 basic groups (Fig. S2, lane 2) or DNAzyme containing 81 basic groups (Fig. S2, lane 3). The two bands in lane 4 belonged to dsDNA obtained by the hybridization of the capture probe and the segment from DNAzyme. The band of dsDNA lay in the above and the band of the segment from DNAzyme lay in below. Because the dsDNA that contains 44 basic groups should migrate faster than the segment from DNAzyme containing 63 basic groups. These results demonstrated that the designed DNAzyme can be cleaved by L-histidine and then the DNAzyme substrate released by L-histidine hybridizes with the capture probe immobilized on the glass slide surface.

3.3. Optimization of experimental conditions

For the purpose of reaching the best sensing performance, the ratio of long and short alkyl-silanes (TEA/DMOAP) was optimized. Gupta groups have proved that the mixed monolayers formed by both long and short alkanes can induce a perpendicular alignment of liquid crystals (Gupta et al., 1998). In this DNAzyme-based LC biosensor, the surfaces are fabricated using TEA/DMOAP-decorated, which not only offer amino groups of TEA for coupling of capture probes, but can also orientate LCs homeotropically. Experiment results show that the number of bright spots in the optical images decrease with the decrease of TEA/DMOAP ratio, and it appears a uniform dark background appearance when the TEA/DMOAP ratio drops to 3:1 (Fig. S3). Thus the TEA/DMOAP ratio of 3:1 was used in the subsequent experiment.

It is necessary to choose a suitable concentration of capture probe (probe 1) to ensure that it is sufficient to hybridize partial substrate strand, which is obtained by cleaving DNAzyme using L-histidine, to cause apparent optical signal but the capture probe change the surface topology as little as possible. It displays that the number of bright spots decreases with the concentration of the capture probe from 1000 nM to 100 nM, and there is almost a uniform dark background appearance when the capture probe concentration reaches 100 nM (Fig. S4). Thus 100 nM of capture probe was used in the subsequent experiment.

3.4. Analytical characteristics

The optical signal characteristics of DNAzyme-based LC biosensor for L-histidine with different concentrations are shown in Fig. 3. Experiment results show that if there is no L-histidine, the optical image reveals uniformly dark texture (Fig. 3a), displaying a low-background for this DNAzyme-based LC sensing system. The domains of birefringent texture in the optical images increase with the concentration of L-histidine from 50 nM to 500 nM as shown in Fig. 3b–f. Moreover, the optical image for 50 nM L-histidine (Fig. 3b) can be easily differed from the dark background. This result suggests that the DNAzyme-based LC sensor system has a good signal-to-background. To our best knowledge, this new

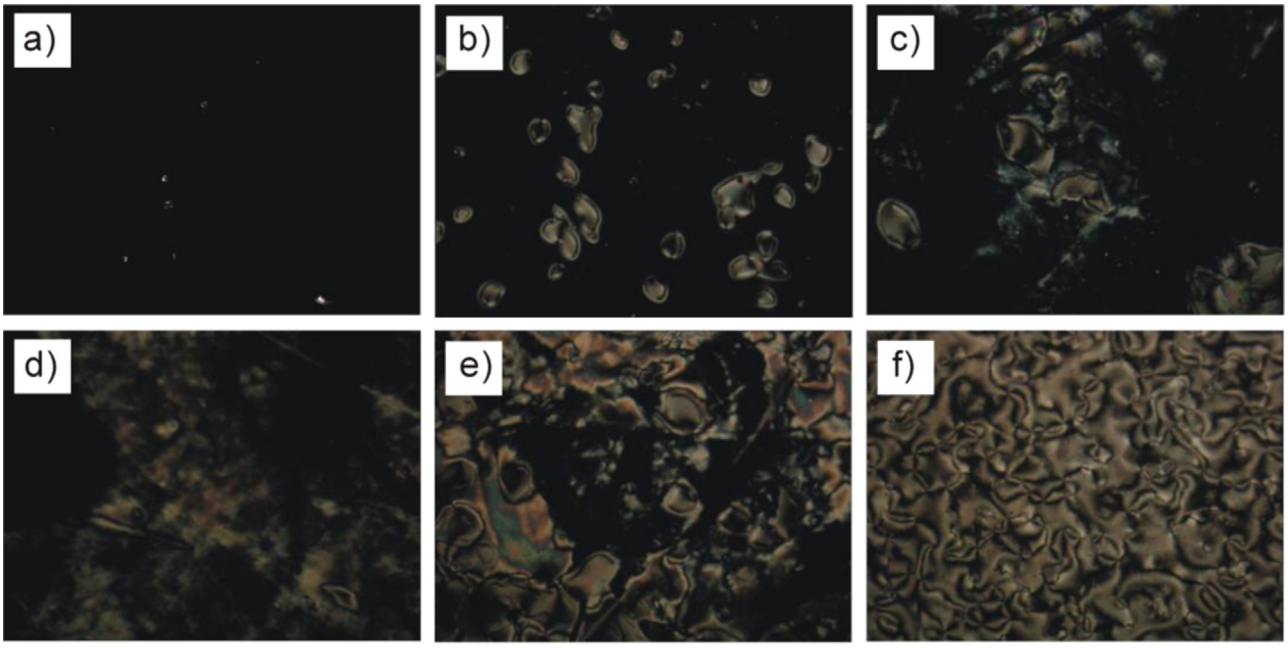


Fig. 3. Typical optical images of the DNAzyme-based LC sensor under crossed polarizers in response to L-histidine at concentrations of (a) 0, (b) 50, (c) 100, (d) 200, (e) 300 nM and (f) 500 nM.

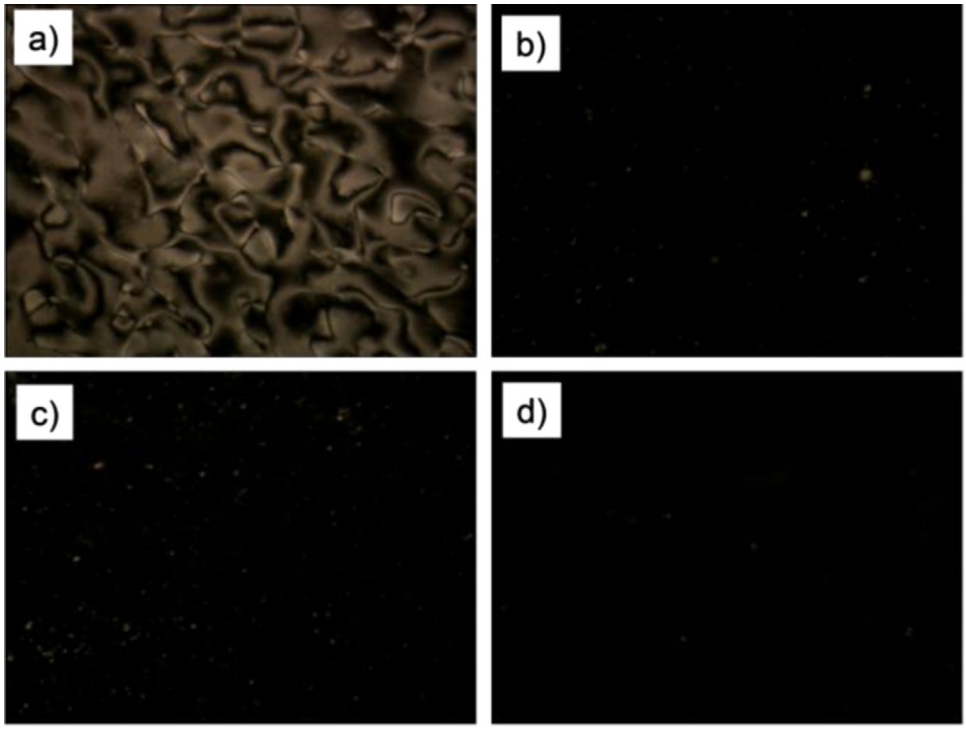


Fig. 4. Selectivity of the DNAzyme-based LC biosensor for L-histidine over other potential interferences: (a) L-histidine (500 nM), (b) phenylalanine (5000 nM), (c) arginine (5000 nM) and (d) lysine (5000 nM).

method is the first example of DNAzyme-based LC biosensor for applying in L-histidine with label-free, none-amplified procedure, but the sensitivity can be comparable to other DNAzyme-based methods for the detection of L-histidine with multi-step amplified procedure. The precision of the DNAzyme-based LC sensor system was achieved by a three-repetitive determination of the same concentrations of L-histidine under the optimum conditions. The resulted outcome shows similar optical images for the same concentration, reflecting this system with good reproducibility for L-histidine.

The selectivity of this DNAzyme-based LC method was investigated by replacing L-histidine (500 nM) with several potential interferences such as phenylalanine, arginine and lysine (5000 nM). As displayed in Fig. 4, an obvious birefringent texture of the optical image is observed for L-histidine, but hardly responded to other amino acids. This observation indicates that the proposed DNAzyme-based LC biosensor at the solid-LC interface has high specificity for L-histidine.

4. Conclusion

So far, most LC-based sensors are developed for the biological macromolecule binding events and few for small molecules, because the optical signal of LC-based sensors is generally thought to be associated with the size and amount of macro-biomolecules, which can greatly change the chemical and topographic of the glass slides, and then disturbing the orientation of LC. In this work, a novel DNAzyme-based LC biosensor has been developed for the high sensitivity detection of small molecule, L-histidine. The L-histidine-mediated formation of ds-DNA on the glass slide surface of LC sensor changed the topography great, causing an evident birefringent texture of optical appearances differed from the background with a dark appearance. The method affords a new DNAzyme-based sensing system without any amplified procedure but with a detection limit of 50 nM for L-histidine. This high sensitivity can be comparable to previously reported multi-step amplified DNAzyme-based sensors (Kong et al., 2011; He et al., 2015). The DNAzyme-based LC sensor system possesses excellent performance such as high selectivity, label-free with high sensitivity and simplicity of the procedure. The proposed strategy is promising for providing a DNAzyme-based LC sensing platform applied in other targets.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.12.107.

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