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Simultaneous detection of dual single-base mutations by capillary electrophoresis using quantum dot-molecular beacon probe

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ABSTRACT

Here a novel capillary electrophoresis (CE) for simultaneous detection of dual single-base mutations using quantum dot-molecular beacon (QD-MB) probe is described. Two QD-MB probes were designed using 585 and 650-nm emitting CdTe QDs which were covalently conjugated to MBs with different DNA oligonucleotide sequences by amide linkage and streptavidin–biotin binding, respectively. The hybridizations of QD-MB probes with different DNA targets were then monitored by CE, and results indicated that the two QD-MB probes specifically hybridized with their complementary DNA sequences, respectively. Target DNA identification was observed to have a high sensitivity of 16.2 pg in CE. Furthermore, the simultaneous detection of dual single-base mutations in a given DNA oligonucleotide was successfully achieved in CE using above two QD-MB probes. This novel CE-assisted QD-MB biosensor offers a promising approach for simultaneous detection of multiple single-base mutations, and exhibits potential capability in the single nucleotide polymorphism (SNP) analysis and high-sensitivity DNA detection.

1. Introduction

DNA is the carrier of genetic information whose mutation often leads to the genetic changes of biological individual. It is significant to detect DNA mutation in biological studies, especially in the area of genetic screening (Whitelaw et al., 2003), genetic disease diagnosis and treatment (Sleeper et al., 2009; Vicart et al., 1998; Qin and Yung, 2009). One of the great challenges in DNA mutation studies is the detection of single-base mutation, especially the simultaneous multiplex single-base mutations detection, due to the high specificity requirement. The emergence of molecular beacon (MB) makes it possible to solve this challenge. MB is a single-stranded oligonucleotide probe which is fast, simple, highly selective and sensitive, and has been widely used in real-time monitoring of polymerase chain reactions (PCRs) (Chen et al., 2000; Chen and Tsourkas, 2009), nucleic acid detection (Santangelo et al., 2004), and DNA–protein interaction study (Li et al., 2000).

MB has a stem-loop structure in which a fluorophore is attached to one end of stem and a quencher is attached to the other end of stem. In comparison with linear oligonucleotide probe, stem-loop shaped MB has high detecting specificity, thus it is also frequently employed in the study of single-base mutation or single nucleotide polymorphism (SNP) (Tyagi et al., 1998; Marras et al., 1999; Liu et al., 2009). Organic fluorescent dyes are usually used as fluorophore in MB-based single-base mutation investigations, but their optical limitations (e.g., spectral cross-talk, low resistance to chemicals and photobleaching) make long-time and multi-target detection more difficult. Even in the developed wavelength-shifting MBs system, the simultaneous detection of multiple targets is hard to realize due to the low differentiation in Stoke's shift for donor–acceptor pairs (10–30 nm), which requires highly sensitive detector with a narrow filter-wavelength window (Tyagi et al., 2000).

In recent years, quantum dots (QDs) have attracted more and more attentions in sensing applications due to their unique optical properties, such as size-tunable photoluminescence spectra, broad absorption, narrow emission and high level of photostability in comparison with conventional fluorescent dyes (Alivisatos, 1996; Chu et al., 2006). Thus, QDs are very suitable for MB-based investigations, and have great potential in the multiplex detection of nucleic acid mutation. Kim group first reported QD-based MB with ability of single base discrimination (Kim et al., 2004). The same group also developed three QD-MB probes which specifically identified the same target DNA, employing green, yellow and orange QDs, and the detection limit of target DNA was observed to be 8 ng (Kim et al., 2007). In another study, QD-MB probe synthesized by self-assembly using a reactive peptidic linker was employed for the specific detection of complementary target DNA (Medintz et al., 2007). Cady et al. (2007) compared the efficiency of different linkage strategies and fluorescence quenchers for creating QD-MB probes in hybridization-based assays. In fact, the greatest hallmark of QDs, in contrast to fluorescent dyes, is its ability to detect multi-targets simultaneously. However, as far as we know, the simultaneous detection for

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Table 1
Alignment of DNA oligonucleotide sequences used in experiment.

Sequence name	Sequence (5′–3′)
MB1	NH_2[a]—**GCGAG**CACCAAACATGATAT**CTCGC**—BHQ2[b]
MB2	Biotin—**GCAGC**ACGTCCTACCCCAAG**GCTGC**—BHQ2
C1	ATATCATGTTTGGTG
C2	ATATCATCTTTGGTG
C3	CTTGGGGTAGGACGT
C4	CTTGGGGAAGGACGT
C5	CTTGGGG**T**AGGACGTATATCAT**G**TTTGGTG
C6	CTTGGGGAAGGACGTATATCATGTTTGGTG
C7	CTTGGGGTAGGACGTATATCATCTTTGGTG
C8	CTTGGGGAAGGACGTATATCATCTTTGGTG

The tail sequences of the MBs were shown underlined and in bold text. For each MB DNA backbone used, the 5′ and 3′ modifications were shown.
[a] Amino group.
[b] Black Hole Quencher 2.

multiple single-base mutations based on QD-MB has never been reported.

On the other hand, the stem-loop structure of MB is only a stable structure in thermodynamics, and could be changed by some factors (e.g., ionic strength and temperature) in the detection of some special systems (Tsourkas et al., 2003; Chen et al., 2007). Thus, a false-positive result would be obtained in the conventional MB method only observing the recovery of fluorophore fluorescence. Capillary electrophoresis (CE) is a high-efficiency separation technique, and has been used in the hybridization analysis of MB with DNA target (Ramachandran et al., 2003; Wang et al., 2009), in which an effective separation of MB-target from unhybridized MB can be achieved. The separation of MB-target avoids the interference of unhybridized MB signal to MB-target ones, which can eliminate the false-positive result completely. In addition, our previous work on QD-based fluorescence resonance energy transfer (FRET) sensor showed that CE is very suitable for high-sensitivity QD-based bioanalysis (Li et al., 2010). Therefore, a novel CE-assisted QD-MB biosensor for the simultaneous detection of dual single-base mutations is presented in this work. Two QD-MB probes with different emission wavelengths and base sequences, were first synthesized using two linkage strategies, respectively. The specificity and sensitivity of QD-MB probes for the detection of complementary target DNA were then analyzed by CE, and the simultaneous detection of dual single-base mutations in a given DNA oligonucleotide was finally performed in CE using the two QD-MB probes.

2. Materials and methods

2.1. Chemicals and reagents

Tellurium power (99.999%), $CdCl_2{\cdot}2.5H_2O$, $NaBH_4$ (96%), L-cysteine (Cys) and reduced glutathione (GSH) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Streptavidin was a product of Promega (Madison, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxylsulfosuccinimide (Sulfo-NHS), N,N,N′,N′-tetramethylethylenediamine (TEMED), ammonium peroxydisulfate (APS) and polyacrylamide (PAA) were from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). All the DNA oligonucleotides used in experiments, including the target DNA and MB DNA modified by a quencher in its 3′ end, were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), and their sequences were shown in Table 1. Microcon molecular weight cut-off (MWCO) spin filters were purchased from Millipore Corporation (Bedford, MA, USA). Uncoated fused-silica square capillary (75 μm square I.D., 365 μm O.D.) was purchased from Yongnian Optical Fibre Factory (Hebei, China). All other materials and reagents were of analytical grade. Ultrapure water (≥18.2 MΩ) from Milli-Q system (Millipore, Bedford, MA, USA) was used for all solutions. The electrophoresis buffers were filtered through a 0.22 μm filter before use.

2.2. Instruments

CE analyses with fluorescence detection were carried out on a home-built system, consisting of a high voltage supply (0–30 kV) (Shanghai Nuclear Research Institute, China), and a IX71 inverted fluorescence microscope (Olympus, Japan) equipped with a 100 W mercury lamp, an excitation filter (BP 420 ± 20 nm), a dichromatic mirror (DM 455), an emission filter (BA 474 nm) and a QE65000 fiber optic spectrometer (Ocean Optics, USA). The fluorescence spectra of two QDs and QD-MB conjugates were measured on a LS-55 spectrophotometer (Perkin Elmer, USA) with a quartz cuvette at room temperature. An excitation wavelength of 420 nm and an emission slit width of 5 nm were used.

2.3. Preparation of CdTe QDs

CdTe QDs capped with stabilizing ligand (GSH or L-Cys) were prepared according to previous literatures (Gao et al., 1998; Wang et al., 2007). In brief, Cd precursor solution was made by mixing a solution of $CdCl_2$ and stabilizing ligand in ultrapure water, and then adjusted to pH 11.2 with 1 M NaOH. Freshly prepared oxygen-free NaHTe solution generated by the reaction of Te powder with $NaBH_4$ (Zhang et al., 2003), was added to nitrogen-saturated Cd precursor solution under vigorous stirring. The resulting mixture solution with the typical molar ratio of 5:6:1 (cadmium:stabilizing ligand:tellurium) was heated to 100 °C and refluxed for proper time to control the size of CdTe QDs. The CdTe QDs obtained were then precipitated with isopropanol, and isolated by centrifugation and decantation to remove free stabilizing ligand. Finally, CdTe QDs were dissolved in 1 mL phosphate buffer (PBS, 10 mM, pH 7.4). The concentrations of CdTe QDs solutions were calculated by a equation proposed by Peng's group (Yu et al., 2003), and the quantum yields (QY) of CdTe QDs were measured by a optically dilute method using rhodamine 6G (whose quantum yield is assumed to be 95% in ethanol) as a criterion (Hua et al., 2006).

2.4. Preparation of QD-MBs Probes

Two linkage strategies were used to conjugate QDs with MBs in our experiments. First, 650-nm emitting GSH-coated CdTe QDs was linked to MB1 by covalent binding in which the carboxyl surface groups of QDs were activated with EDC and Sulfo-NHS, and allowed to react with the 5′ amino groups on MB1. Briefly, 30 μL EDC PBS solution (5 mg/mL) and 20 μL Sulfo-NHS PBS solution (1.5 mg/mL) were added to 30 μL GSH-coated CdTe QDs PBS solution (45 μM), stirred for 0.5 h at room temperature. Then 200 μL MB1 PBS solution (100 μM) was added, and the mixture was allowed to react for 2 h at room temperature with continuous gentle stirring. Unreacted MB1, free EDC and Sulfo-NHS were removed from the QD-MB1 by spin filtration in a Millipore Microcon 50 kDa MWCO spin filter. Reactions were spun at 8000 × *g* for 5 min, and the retentate (QD-MB1) was resuspended in 400 μL PBS while the flow-through was discarded. This was repeated two times and the QD-MB1 probe was finally resuspended in 300 μL PBS.

In order to achieve the specific conjugation of QDs with biotinylated MB2, 585-nm emitting Cys-coated CdTe QDs was firstly covalently bound to streptavidin by a similar procedure as QD-MB1 combination. Approximately 500 pmol of Cys-coated CdTe QDs were mixed with 390 nmol of EDC and 130 nmol of Sulfo-NHS in 300 μL of PBS, stirred for 0.5 h at room temperature. Then 100 μL streptavidin PBS solution (5 mg/mL) was added, and the mixture was allowed to react for 1.5 h at room temperature

and washed with PBS in a Millipore Microcon 100 kDa MWCO spin filter. The retentate (QD-streptavidin) was resuspended in 200 μL PBS. Subsequently, the QD-MB2 probe was obtained by the streptavidin–biotin interaction. Briefly, 200 μL MB2 PBS solution (100 μM) was added to the QD-streptavidin PBS solution obtained above, and the mixture was incubated for 1 h and washed two times with PBS in a Millipore Microcon 100 kDa MWCO spin filter. The final retentate (QD-MB2) was resuspended in 300 μL PBS. The two QD-MB probes were stored in the dark at 4 °C and were used within 1 month of their initial synthesis.

2.5. CE procedure

CE analyses were carried out on a home-built system. A capillary was fixed on the detecting platform of an inverted fluorescence microscope, and a detection window was simply made by burning a specific length of polyimide coating of capillary above the objective lens of microscope. A 100 W mercury lamp was used as excitation light source. After passing through the excitation filter (BP 420 ± 20 nm) and reflected by a dichromatic mirror (DM 455), the excitation light (420 ± 20 nm) was focused on the detecting window by an objective lens to excite the samples moving through it. The fluorescence emitted from the samples were collected by the same objective, passed through the dichromatic mirror and an emission filter (BA 474 nm), and finally recorded by a fiber optic spectrometer. For the suppression of electroosmotic flow (EOF) and absorption of samples on the capillary inner surface, the inside wall of the capillary was coated with PAA according to the previously reported procedure (Wang et al., 2008). The capillary was 60 cm long with an effective length of 35 cm from the inlet to the detection window. Reversed polarity conditions, i.e. the anode at the capillary outlet end, were used for all separations. The samples were electrokinetically introduced to the capillary. CE analysis was carried out at room temperature. Before analysis, the capillary was rinsed and equilibrated with electrophoresis buffer (25 mM $Na_2B_4O_7$, pH 9.0) for 15 min. Between each run, the capillary was washed by pure water and electrophoresis buffer for 10 min in sequence to ensure the reproducibility.

3. Results and discussion

3.1. Principle of CE-assisted QD-MB biosensor for simultaneous detection of dual single-base mutations

In order to demonstrate the utility of CE-assisted QD-MB biosensor for the multiplex detection of single-base mutation, it was used in assays that simultaneously detect two different single-base mutations in our experiment. As an example, two single-base mutations in a constructed DNA oligonucleotide (C5 in Table 1, the bases at mutation sites were shown underlined and in bold text) were studied, in which an adenosine residue can substitute for a thymidine residue at position T, and a cytidine residue can substitute for a guanosine substitute at position G. Thus, four different DNA sequences can occur: ··T··G··, ··A··G··, ··T··C·· and ··A··C··. In order to uniquely detect the two single-base mutations, two QD-MB probes were constructed. As shown in Table 1, the 15-base loop sequence of QD-MB1 was complementary to the 3′ end sequence of C5 in which mutation position G was existed, and the 15-base loop sequence of QD-MB2 was complementary to the 5′ end sequence of C5 in which mutation position T was existed. Fig. 1 shows a schematic of CE-assisted QD-MB biosensor for the simultaneous detection of single-base mutation conditions at position T and G. The two QD-MB probes were first mixed and allowed to react with mutated C5 needed to detect in hybridization buffer, and the mixture was then analyzed by CE. After analyzing the electropherogram

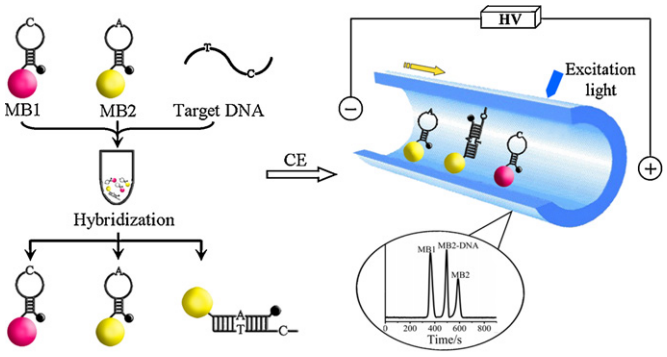

Fig. 1. The schematic of CE-assisted QD-MB biosensor for the simultaneous detection of single-base mutation conditions at two position.

obtained, the mutation condition of C5 was finally determined by the occurrence of hybridized electrophoresis peak.

3.2. Characterization of QDs and QD-MB probes

The fluorescent emission wavelength and intensity of QDs after conjugation with MBs were first investigated. In Fig. S1 (Supporting Information) the fluorescent emission wavelength of Cys-coated CdTe QDs (QY = 28%) was 585 nm (Fig. S1a), and shifted to 593 nm after conjugation with streptavidin (Fig. S1b) and MB2 (Fig. S1c), while its fluorescent intensity was reduced by about 60%. Similarly, the fluorescent emission wavelength of GSH-coated CdTe QDs (QY = 18%) had a slightly red shift from 650 (Fig. S1d) to 653 nm (Fig. S1e) after conjugation with MB1, while its fluorescent intensity was decreased by about 40%. The reason for the red shift of QDs' fluorescent emission wavelength was probably due to the increment of its size after conjugation. The decrease of QDs' fluorescent intensity after conjugation was mainly attributed to the occurrence FRET between QDs and quencher in QD-MBs, whereas the loss or aggregation effects of QDs might happen during the conjugation procedures. The formation of stem-loop structure in QD-MBs shortened the distance between quencher and QDs, resulting in the occurrence of FRET between them and the fluorescence quenching of QDs. The decrease degree of QDs' fluorescent intensity was mainly related to the quenching efficiency of quencher on it. The quencher used in our experiment was Black Hole Quencher 2 (BHQ2) that is a common quencher having relatively high quenching efficiency over a broad range of wavelengths (Marras et al., 2002), and its maximal absorption wavelength was around 580 nm, resulting in the higher quenching efficiency on 585-nm emitting Cys-coated CdTe QDs (60%) than 650-nm emitting GSH-coated CdTe QDs (40%). In addition, the characterization of QDs and QD-MB conjugates were also studied by CE. The electrophoresis mobilities of two QDs and QD-MB conjugates were observed to be different from their electropherograms (Fig. S2 in Supporting Information), which was directly related to the difference of their charge-to-size ratio (described detailedly in the Supporting Information).

3.3. CE analysis for hybridization of QD-MB probes with targets

The performances of two QD-MB probes were tested by CE with regard to their hybridization with the complementary target DNA (CT) and single-base mutated target DNA (SMT), respectively. In the electropherograms for the hybridization mixture of QD-MB probes and CT (curve c, Fig. 2A and B), in addition to the electrophoresis peaks corresponding to QD-MBs (peak 2), hybridized electrophoresis peaks (peak 3) with higher fluorescent intensities and shorter migration times were also found. The charge-to-mass ratio of QD-MBs was increased after hybridiza-

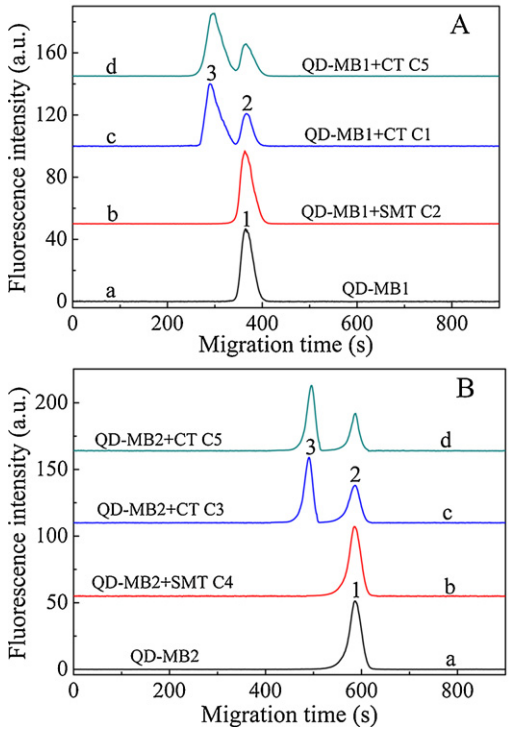

Fig. 2. Electropherograms for hybridization analysis of 120 nM GSH-coated CdTe QD-MB1 (A) and Cys-coated CdTe QD-MB2 (B) with 336 nM different DNA targets. A solution containing 20 mM Tris–HCl, 50 mM KCl and 5 mM $MgCl_2$ at pH 8.0 was used as the hybridization buffer, and the hybridization temperature was 20 °C. Coated capillary with 35 cm effective (60 cm total) length and 75 μm I.D. was used. 25 mM $Na_2B_4O_7$ (pH 9.0) was used as running buffer. Applied voltage was −18 kV, and electrokinetic injection was carried out at −14 kV for 25 s.

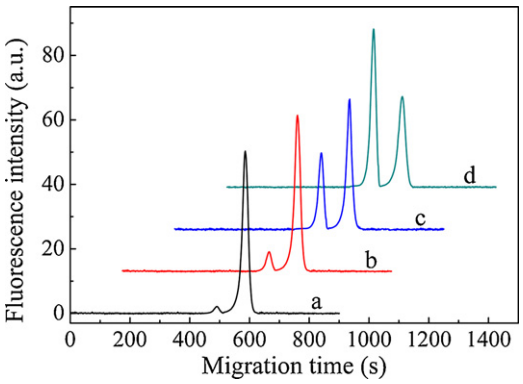

Fig. 3. Electropherograms for the hybridization mixture of 120 nM Cys-coated CdTe QD-MB2 probe and different amounts of C5: (a) 14 nM; (b) 42 nM; (c) 168 nM; (d) 336 nM. Other conditions were as described in Fig. 2.

tion, which was the reason for the shorter migration times of hybridized electrophoresis peaks. In addition, the occurrence of hybridized electrophoresis peaks with higher fluorescent intensities indicated that the hybridization of QD-MB probe with CT was occurred in the mixture, leading to the change of QD-MB stem-loop structure and the fluorescence enhancement of QD-MB probes (namely the fluorescence recovery of QDs binding with target). Moreover, the fluorescence enhanced level of QD-MB probe in QD-MB-target hybrid ($S_{peak\,3}/(S_{peak\,1} - S_{peak\,2})$, where S stands for the area of corresponding electrophoresis peak) in Fig. 2B was found higher than that in Fig. 2A, which was mainly due to the different quenching efficiency of BHQ2 on Cys- and GSH-coated CdTe QDs before hybridization. In the electropherograms for the hybridization mixture of QD-MB probes and SMT (curve b, Fig. 2A and B), only the electrophoresis peaks corresponding to QD-MBs were found, and their fluorescent intensities were not changed compared with those of electrophoresis peaks for QD-MBs sample (curve a, Fig. 2A and B) having the same concentration with the hybridization mixture, indicating that no hybridization was occurred in the mixture. Namely the two QD-MB probes we obtained have the ability of single base discrimination. Furthermore, the hybridizations of QD-MB probes with long-chain complementary target DNA (C5, Table 1) were also explored. The sequence of C5, as shown in Table 1, could be both complementary to the loop sequences of two QD-MB probes. Results showed that the electropherograms for the hybridization mixture of QD-MB probes and C5 (curve d, Fig. 2A and B) were similar with those of short-chain complementary target DNA (curve c, Fig. 2A and B), indicating that the chain length of complementary target DNA had little effect on the hybridization results (e.g., the migration time and intensity of hybridized electrophoresis peak). The chain lengths of targets used in our experiment were all short, and the situation would be different for the detection of target with long sequence (e.g., 1 kb). Some complicated structures are always formed in the single-stranded target with long sequence due to the partial hybridization. These structures sometimes can block the hybridization between QD-MB probe and target, leading to the instability of probe/target hybrid during CE. Therefore, the target with long sequence should be digested by specific restriction enzyme before detection.

To further investigate the hybridization of QD-MB probe with complementary target DNA, the electropherograms for the hybridization mixtures containing fixed quantity of Cys-coated CdTe QD-MB2 and different amounts of complementary target DNA (C5 in Table 1) were studied. As shown in Fig. 3, the electrophoresis peak for the QD-MB2 probe gradually decreased with the increasing of C5 amount (Fig. 3a–d), while the electrophoresis peak for the hybridization product QD-MB2–C5 gradually increased. This phenomenon indicated that more QD-MB2–C5 binding was formed with the increasing of C5 concentration (shown clearly in the Supporting Information Fig. S3). Furthermore, it was also found that C5 as low as 16.2 pg (14 nM) was identified (Fig. 3a). Compared to the literature for the detection of target DNA based on QD-MB with a detection limit of 8 ng (Kim et al., 2007), our CE-assisted QD-MB biosensor exhibited much higher sensitivity. Moreover, it is believed that the detection sensitivity of our method can be greatly increased by introducing laser into our CE system as the excitation light source. The improved detection limit obtained in our method may be attributed to the efficient separation of QD-MB-target hybrid from free QD-MB probe, which avoids the interference of unhybridized QD-MB signal and makes the detection more sensitive. Certainly, the low sample consumption property of CE also contributes to the improved mass detection limit.

3.4. The simultaneous dual single-base mutations assay based on CE-assisted QD-MB method

The two QD-MB probes were subsequently used together in the simultaneous dual single-base mutations detection of C5 in CE after their ability of single base discrimination were proved. The sequence of C5, as mentioned earlier, could be divided into two parts which are complementary to the loop sequences of two QD-MB probes. Therefore, C5 can hybridize to both of the two QD-MB probes in the condition of no mutation. However, if mutation happened at position T or G, the hybridization of C5 with corresponding QD-MB probe would not occurred, and the corresponding hybridized electrophoresis peak would not be observed in the electropherogram for the hybridization mixture of two QD-MB probes and mutated C5, due to the single base discrimination ability of QD-MB probes. Thus, the mutation condition of C5 can be determined by analyzing the occurrence of hybridized electrophoresis peak in

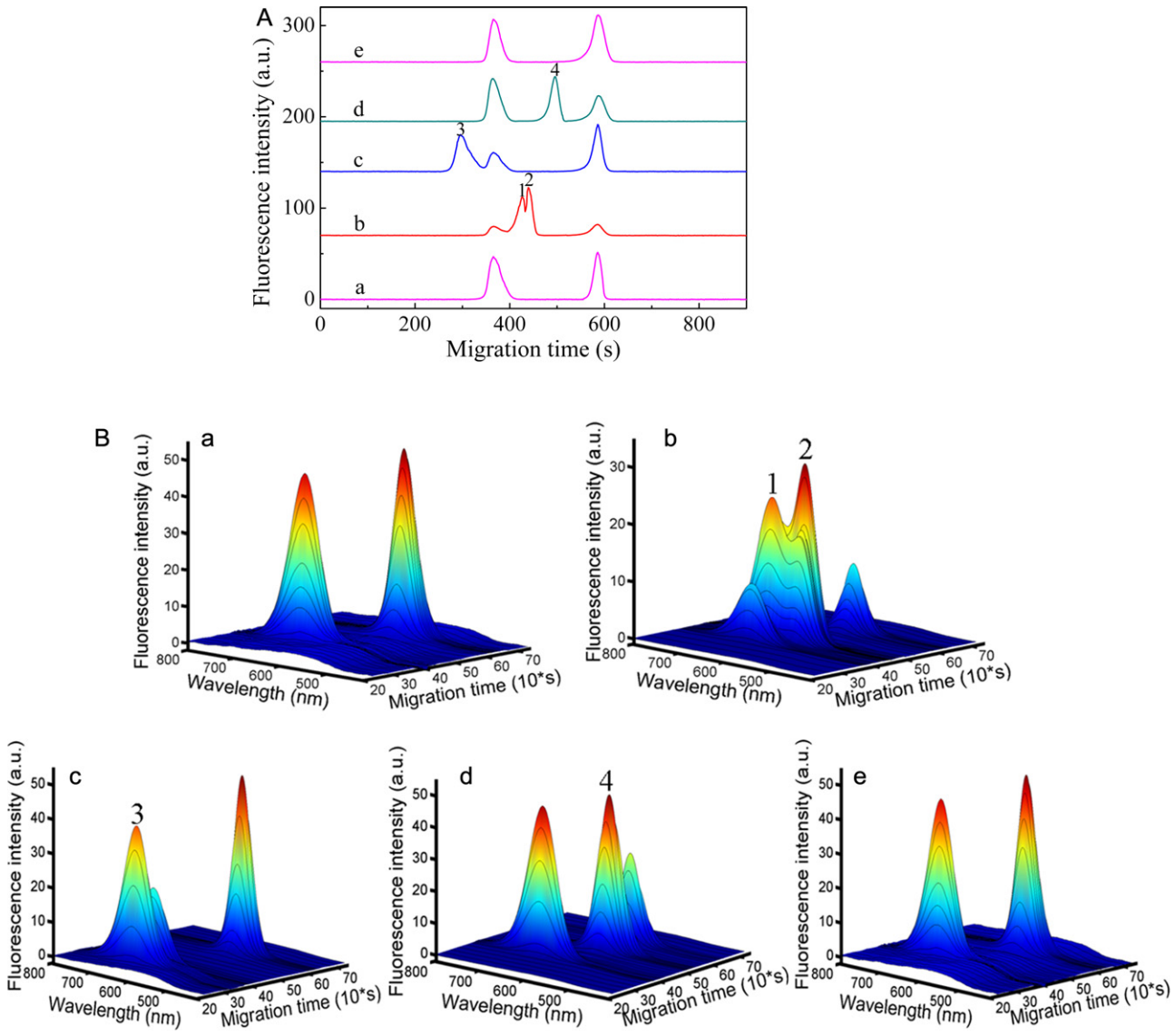

Fig. 4. Electropherograms (A) and corresponding 3-D fluorescence spectra (B) for the hybridization mixture of two QD-MBs and different mutation forms of C5. Sample: (a) 120 nM QD-MB1 + 120 nM QD-MB2; (b) 120 nM QD-MB1 + 120 nM QD-MB2 + 336 nM C5; (c) 120 nM QD-MB1 + 120 nM QD-MB2 + 336 nM C6; (d) 120 nM QD-MB1 + 120 nM QD-MB2 + 336 nM C7; (e) 120 nM QD-MB1 + 120 nM QD-MB2 + 336 nM C8. Other conditions were as described in Fig. 2.

the electropherogram for the hybridization mixture of two QD-MB probes and C5.

In order to analyze the mutation of C5 more accurate, two control electropherograms were analyzed in advance. First, the electropherogram for the mixture of two QD-MB probes was studied. As shown in curve a (Fig. 4A), two electrophoresis peaks were found and were easily assigned to the GSH-coated CdTe QD-MB1 and Cys-coated CdTe QD-MB2 probe respectively, according to their migration times and the 3-D fluorescence spectra (Fig. 4B, a) corresponding to curve a (Fig. 4A). In addition, the electropherogram for the mixture of two QD-MB probes and normal C5 was also investigated. As shown in curve b (Fig. 4A), in addition to the electrophoresis peaks with reduced intensities corresponding to the two QD-MB probes, two hybridized electrophoresis peaks (peaks 1 and 2) close to each other were also found. From the 3-D fluorescence spectra (Fig. 4B, b) corresponding to curve b (Fig. 4A), it was found that the fluorescence signals of two QDs (GSH- and Cys-coated CdTe QDs) were simultaneously detected within the migration time range (390–460 s) of peaks 1 and 2. This phenomenon indicated that the corresponding hybridization products of peaks 1 and 2 contained two QD-MB probes, in other words, the peaks 1 and 2 represented the QD-MB1/C5/QD-MB2 hybrids. From the 3-D fluorescence spectra (Fig. 4B, b), it was also found that the signal ratio of two QDs fluorescence was different within the migration time range of peaks 1 and 2, indicating that the two QD-MB1/C5/QD-MB2 hybrids contained different proportions of two QD-MB probes and this could be the reason for their different migration times.

Subsequently, the attempt to determine the mutation condition of C5 was performed by analyzing the electropherogram for hybridization mixture of mutated C5 (C6, C7 and C8 in Table 1) and two QD-MB probes. In the curve c (Fig. 4A), in addition to the electrophoresis peaks corresponding to two QD-MB probes, a peak (peak 3) with shorter migration time was also found. The peak 3 can be assigned to the hybridization product of QD-MB1 probe, according to its migration time and the 3-D fluorescence spectra (Fig. 4B, c) corresponding to curve c (Fig. 4A). This phenomenon indicated that a single-base mutation occurred at position T of C5 which blocked the hybridization of QD-MB2 with C5. Therefore, it can be inferred that the sequence used in curve c (Fig. 4A) should be ··A··G··. Similarly, besides the electrophoresis peaks for two QD-MB probes, only the hybridized peak (peak 4) correspond-

ing to the hybridization product of QD-MB2 probe was observed in the curve d (Fig. 4A), indicating that a single-base mutation occurred at position G and the sequence used here should be ··T··C··. Moreover, no hybridized electrophoresis peak was found in the curve e (Fig. 4A), indicating that single-base mutation occurred both at position T and G and the sequence used was presumed to ··A··C··. By comparing these inferred sequence results with the DNA oligonucleotides used in the actual situation, it was easy to found that our inferred sequences (··A··G··, ··T··C·· and ··A··C··) were completely consistent with that of DNA oligonucleotides (C6, C7 and C8) used in the curves c–e (Fig. 4A). Therefore, these results indicate that our CE-assisted QD-MB method is suitable for the high-accuracy simultaneous detection of dual single-base mutations.

In comparison with the popular Affymetrix microarray platform (Genome-Wide Human SNP Array 6.0) for the study of single-base mutations or SNPs, our method possesses the advantages of high sensitivity, low sample consumption, low cost, simple operation and sample preparation, which can significantly contribute to the study of SNPs. But on the other hand, over 1.8 million SNPs can be detected simultaneously in the Affymetrix platform due to its high spatial resolution, which is difficult for our CE-based system. In our method, the detection number of single-base mutation achieved simultaneously is mainly dependent on the resolution of multicolor QD-MB conjugates in CE. In addtion, therefore, all the strategies which can improve the resolution of multicolor QD-MB conjugates, are useful and should be considered for further detection of multiple single-base mutations. Since the loop structure of MB used contains 15 bases, two MB-QD probes are sufficient for the simultaneous detection of two single-base mutations separated by 14 bases in our experiments. However, if the distance between the two single-base mutations is less than 7 bases, four MB-QD probes will be needed to achieve simultaneous detection. In other words, the spatial resolution of single-base mutations (or SNPs) is closely related to the detection in our method, and the CE-assisted QD-MB method may be extend to allow for the spatial resolution of various single-base mutations (or SNPs) to a certain extent.

4. Conclusions

In this study, the simultaneous detection of dual single-base mutations in a given DNA oligonucleotide was successfully achieved in CE using two QD-MB probes with ability of single base discrimination. These two QD-MB probes were elaborately designed by conjugating two probe-sequenced MBs on CdTe QDs with two colors. In comparison with conventional MB technique, this CE-assisted QD-MB method are high sensitive (16.2 pg), free from false-positive result, and easy for simultaneous multiplex detection. In addition, only trace amount of sample (about 0.12 μL) is consumed, which is important for bioanalysis since it can greatly reduce the cost of experiment. This novel CE-assisted QD-MB biosensor can be easily extended to the SNP analysis and is promising to be applied in the study of high-sensitivity DNA detection.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.10.002.

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