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A microchip electrophoresis-based assay for ratiometric detection of kanamycin by R-shape probe and exonuclease-assisted signal amplification

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ABSTRACT

Excessive intake of kanamycin (KANA) can cause some serious drug-resistant diseases, so it is urgent to develop some accurate and rapid analytical methods for monitor KANA residues in foodstuffs with complex matrix. Recently, many ratiometric assays were reported to be capable of overcoming matrix interference. Herein, a ratiometric and homogeneous assay for KANA detection based on microchip electrophoresis (MCE) was developed. First, by one single strand DNA (S-DNA) and one hairpin DNA (H-DNA), a novel R shape DNA probe (R-DNA) was prepared. After the probe was incubated with KANA, the S-DNA-KANA complex was formed, and H-DNA was released. Moreover, in the presence of exonuclease I (Exo-I), S-DNA-KANA complex would be digested to release the captured KANA for triggering target recycling and signal amplification. With the reaction going on, the fluorescence intensity of H-DNA (I_H) increased and that of R-DNA (I_R) decreased. They can be separated at different voltage intensities and converted to fluorescent signals for signal readout by MCE. The signal ratio of I_H/I_R was found to be linear toward target from 0.5 pg mL^{-1} to 10 ng mL^{-1}, and the limit of detection was 150 fg mL^{-1}. Moreover, it was successfully employed for KANA detection in milk and fish samples with consistent results of enzyme linked immune sorbent assay (ELISA). The R-DNA probe can quantitatively convert the amount of target to the intensity of DNA without label by MCE, and achieved exonuclease assisted signal amplification in homogenous solution. It was valuable to detect antibiotics residues in foodstuff with complex matrix. This approach broadened the application field of MCE to detect antibiotics without derivatization, which provided a promising platform for rapid screening of antibiotic residues in food.

1. Introduction

Natural or synthetic origin antibiotics are able to kill or inhibit the growth of micro-organisms [1]. However, rampant use of antibiotics can make some serious drug-resistant diseases, e.g. resistant superbugs in human being [2]. Some antibiotics like kanamycin (KANA) may enter to the human body through water and food. Thus it is urgent to develop some effective, accurate analytical methods for rapid antibiotics residues detection in foodstuffs. There are many kinds of screening methods including the conventional high-performance liquid chromatography (HPLC) [3], enzyme-linked immunosorbent assay [4] and gas or liquid chromatography coupled with mass spectrometry (GC-MS and LC-MS) [5,6], electrochemical immunosensor [7], aptamer-based sensor (aptasensor) [8], etc. But, these assays still remained some shortcomings. For examples, LC-MS has high sensitivity and accuracy, but it is suffered from some defects such as expensive instrument, tedious pretreatment process, which makes it not suitable to on-site screening of antibiotics [9]. Immunoassay is simple and practical, but the antibody is expensive, liable to deactivate and can bring false positive results in some samples with complex matrix [10]. Microchip electrophoresis (MCE) is a high-throughput separation and analytical method which is similar to capillary electrophoresis (CE) with distinctive advantages of miniature and integrated structure, high automation efficiency and tiny sample consumption [11]. Thus, it has been widely adopted in biochemical analysis and can be acted as a candidate technique for developing screening method [12,13]. In recent years, there are some reports for antibiotics detection using MCE [14–16]. For example, Henry et al. successfully separated within 1 min penicillin or ampicillin by MCE with pulsed amperometric detection, and its detection limit reached to 5 μM [17]. Ding et al. developed a MCE-based

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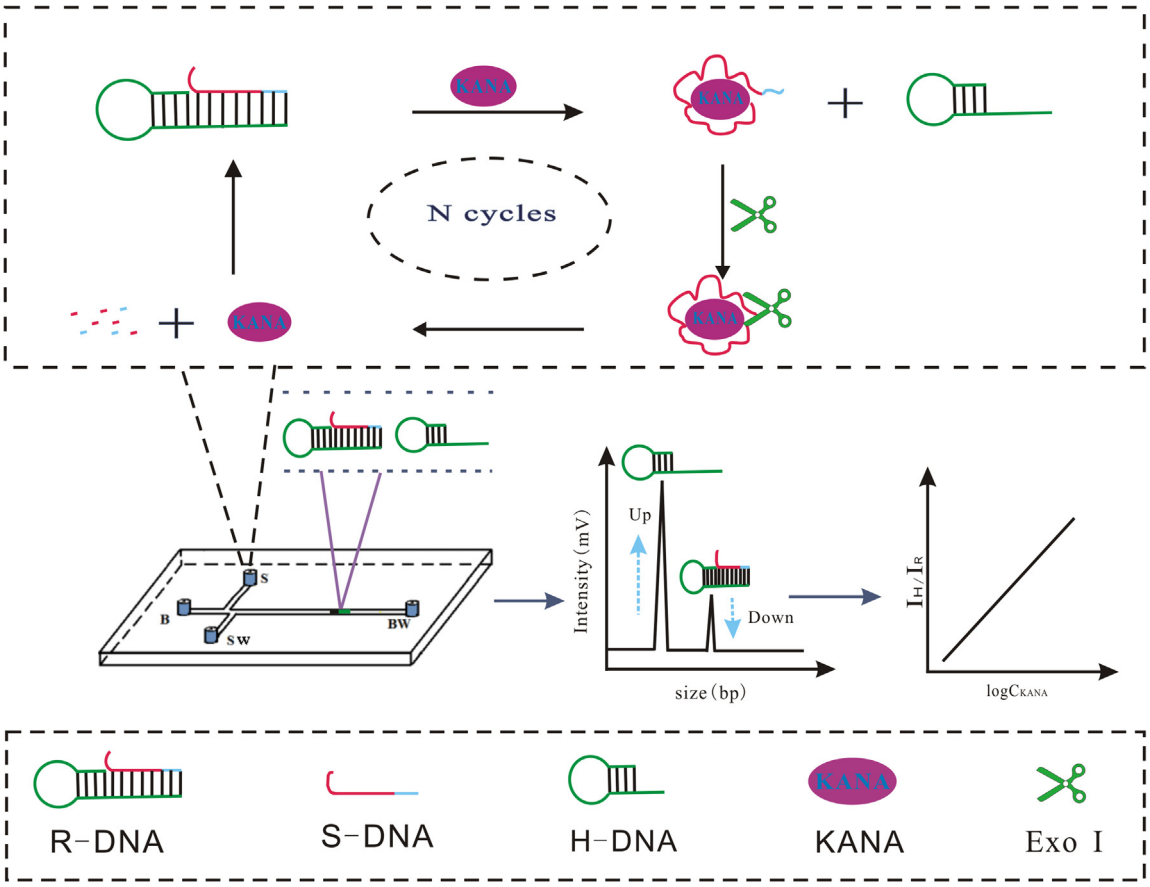

Scheme 1. Schematic representation of detection of Kanamycin (KANA) based on R shape DNA probe with Exo-I assisted target recycling amplification.

assay with amperometric detector to separate five aminoglycoside antibiotics (AGs) in spiked milk samples within 3 min [18]. He et al. developed an assay that can separate anthracycline antibiotics, doxorubicin and daunorubicin in 60 s [19]. However, most of the MCE chips were specially designed and focused on charged analytes with intrinsic luminescence or electroactivity [20,21]. For the electroneutral and non-fluorescent antibiotics, such as Kanamycin (KANA) chloramphenicol (CAP) [22,23], the related studies are seldom reported because the target cannot be easily separated and detected without derivatization. Thus, the previous works were limited the further application of MCE in antibiotics detection.

As it is well known, aptamer is a kind of DNA or RNA fragment with certain bases sequence that can specifically capture the target with high affinity. Moreover, different lengths of aptamers can be easily separated and detected at different intensities of current and then convert to fluorescent signals for signal readout by MCE. Herein, we proposed a detection assay using a novel aptamer probe for accurate and rapid screening of KANA based on MCE. The Scheme 1 was shown as following: firstly, one kind of R shape DNA probe (R-DNA) was constructed by one single strand DNA (S-DNA, contains aptamer fragment of KANA and irrelevant sequence) and one hairpin DNA (H-DNA, it was partially complementary with 3′ -end of S-DNA). After the KANA and R-DNA solution were incubated, the probe was produced S-DNA-KANA complex to release H-DNA. The S-DNA-KANA complex was easily digested by Exonuclease I (Exo-I) for the next target recycling [24,25]. Because of the blocked 3′ -end, R-DNA and the released H-DNA could exist stability with the introduction of Exo-I and they can be easily separated and detected by MCE owning to their different length of base-pairs. Recently, the ratiometric method has been proven to be a good choice to improve the practical applications of sensors due to the existence of matrix interference [26]. And the increased H-DNA and reduced R-DNA fluorescence intensities ratio (I_H /I_R) was quantified for the corresponding target concentration with the cycling going on [27]. The assay was employed in milk and fish samples detection, and the results were compared with that of ELISA.

The main advantages of this aptamer system are listed as following: firstly, a novel R-shape aptamer probe is designed to specifically convert the analytes into DNA fragment in a homogeneous system. The probes and products are separated easily by MCE for DNA analysis. Based on the signal conversion mode, the application of antibiotics detection based on MCE has been broadened greatly. Secondly, this developed aptamer system provides a ratiometric analytical strategy [28–30]. It is valuable to detect antibiotics residues in foodstuff with complex matrix. Thirdly, the assay may obtain a high sensitivity based on exonuclease-assisted target recycling for signal amplification. Moreover, the assay was successfully employed in KANA detection in several fish and milk samples.

2. Experimental

2.1. Reagents and apparatus

The sequence oligonucleotides used in this strategy were shown in Table S1. All the DNA molecules were custom-designed and then synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). Kanamycin (KANA), chlortetracycline (CTC), tetracycline (TET), chloramphenicol (CAP), erythromycin (ERY) and gentamicin sulfate (GS) were purchased from Sigma-Aldrich Co., Ltd (Milan, Italy). Sodium nitroprusside (SNP, $Na_2[FeII(CN)_5NO]$), zinc sulfate, anhydrous sodium sulfate and ethyl acetate(99.8%) were purchased from Sigma-Aldrich Co., Ltd (Milan, Italy). Exonuclease I (Code No. 2650 A) and its buffer were purchased from Takara