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Detection of DNA utilizing a fluorescent reversible change of a biosensor based on the electron transfer from quantum dots to polymyxin B sulfate

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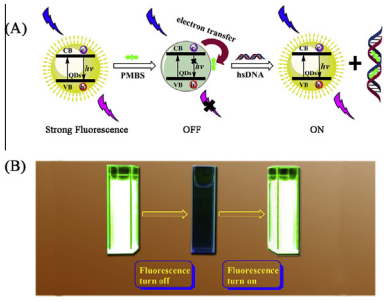
GRAPHICAL ABSTRACT



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ABSTRACT

A fluorescent "turn off–on" pattern for the detection of herring sperm DNA (hsDNA) had been designed through utilizing the interaction between polymyxin B sulfate (PMBS) and hsDNA as an inherent performance and the fluorescent transformation of glutathione (GSH)-capped CdTe quantum dots (QDs) as an external manifestation. Due to the occurrence of the photoinduced electron transfer from the QDs to PMBS, the fluorescence of GSH-capped CdTe QDs could be effectively quenched by PMBS, causing the system into "off" state. With the addition of hsDNA, the quenched fluorescence of GSH-capped CdTe QDs could be restored for the reason that PMBS embedded into hsDNA double helix structure to form new complex and peeled off from the surface of GSH-capped CdTe QDs, leading the system into "on" condition. Corresponding experimental results illustrated that the relative recovered fluorescence intensity of GSH-capped CdTe QDs–PMBS system was near proportional to the concentration of hsDNA within the range of 0.059–15.0 μg mL^{-1}. This proposed method demonstrated a good linear correlation coefficient of 0.9937 and a detection limit (3 σ/K) of 0.018 μg mL^{-1} for hsDNA. This dual-directional fluorescent biosensor overcame the selectivity problem commonly existed in the traditional mono-directional fluorescence detection mode and owned perfect analysis applications in biochemical DNA monitoring.

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1. Introduction

Deoxyribonucleic acid (DNA) plays an extremely important role in the process of human life, which serving as the carrier of genetic information and gene expression of the material basis. The development of novel effective probes for the detection of DNA and the interactions of drugs with DNA have been the focuses, on account of they may be conducive to realizing the effective mechanism of drugs and the design of new specific DNA-targeted drugs [1–3]. DNA with their evenly stacked base pairs and shallow (minor) and deep (major) grooves are attractive targets for some

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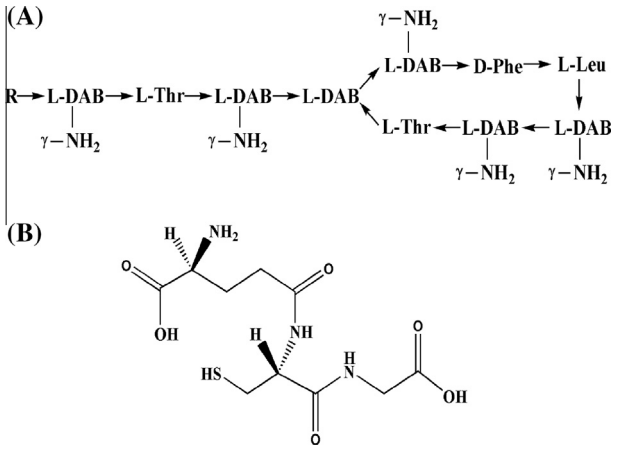

Fig. 1. Chemical structures of (A) polymyxin B (DAB, α, λ-diaminobutyric acid; Thr, threonine; Phe, phenylalanine; Leu, leucine) and (B) glutathione.

drug molecules. The interaction between DNA and drug can cause changes to the structure of DNA, thereby affecting the function of gene-specific regulation and expression in pharmacological activity. Several methods for detection trace amounts of DNA have been established, including fluorescence [4], chemiluminescence [5], electrochemistry [6], and so on. Among them, fluorescence based analytical method are used extensively for its handy, rapid, selective, and the reagents are easily obtainable.

Polymyxin B sulfate (PMBS, molecular structure given in Fig. 1(A)) is an antibiotic widely used in the treatment of infections caused by gram-negative bacteria, which is due to its stronger antibacterial capacity. Previous researches have shown that PMBS, it by itself contains two Thr-OH and five DAB with carbonyl group, can invade lipoprotein through the molecule lipophilic groups and damage the shielding effect of the cell plasma membrane, causing the huge loss of cell sap and the bactericidal effect [7,8]. Nevertheless, whether it can combine with DNA and affect the structure of DNA still unclear.

Among the rich family of inorganic nano-scale building blocks, quantum dots (QDs) attract the attentions of many researchers in recent years. QDs have unique functional and structural properties, such as small size, large absorption cross sections, narrow and Gaussian emission spectra, size- and composition-tunable emission and high photobleaching threshold [9,10]. All of these properties provide important advantages for QDs becoming excellent candidates in the development of novel and sensitive sensors in current researches. Earlier studies related to the interactions of QDs with some compounds have revealed that the interactions make a profound effect on the photophysical properties of QDs [11,12].

While more and more researches have been focusing on the exploration of QDs utilized as fluorescent "turn-off" sensors, very few studies about QDs-based fluorescent "turn off–on" sensors are reported [13,14]. For example, Fang et al. developed a graphene oxide (GO)-based multicolor fluorescent "turn-off" probe to detect DNA targets in homogeneous solution which was depending on the interaction between GO and DNA molecules [15]. Since a variety of factors rather than analytes can induce the ultimate fluorescence "off" state, a fluorescent "turn off–on" model is designed to overcome this problem and effectively lessen the chance of false judgment. Meanwhile, this "turn off–on" pattern is amenable to complex situations, for instance, the simultaneous exclusively detections of sorts analytes [16]. In this study, we had realized a new fluorescent sensor to detect hsDNA (herring sperm DNA) based on the reversible "off–on" fluorescence change of GSH capped-CdTe QDs. PMBS was utilized as it could efficiently quench the high fluorescence of GSH capped-CdTe QDs which causing the system into "off" state. This approach was relied on the electrostatic interaction between the oppositely charged substances of PMBS and GSH-capped CdTe QDs, giving rise to the electron transfer from the photoexcited QDs to PMBS. On the other hand, the high affinity of PMBS to hsDNA enabled PMBS to intercalate into the hsDNA double helix structure and peel off from the surface of GSH-capped CdTe QDs. Therefore, this intercalation reaction hindered the proceeding of the electron transfer from the QDs to PMBS, while recovering the fluorescence of the QDs and leading the system into "on" condition. The principle of this designed fluorescent biosensor for the determination of hsDNA was illustrated in Scheme 1. This dual-directional regulation fluorescence mode of GSH-capped CdTe QDs resolved the selectivity problem existing in traditional mono-directional fluorescence detections. Since it required no modification or coupling of QDs, the drug molecular and DNA were all under the original state, the developed fluorescent "turn off–on" biosensor exhibited the advantages of fast, simple, sensitive and selective for hsDNA monitoring.

2. Materials and methods

2.1. Apparatus

A Hitachi F-2500 fluorospectrophotometer (Tokyo, Japan) was used for measuring the fluorescence with the excitation wavelength of 350 nm and the scattering intensities. The absorption spectra were recorded by making use of a UV-2450 spectrophotometer (Tianmei Corporation, Shanghai, China). JEOL JEM-2100 transmission electron microscopy (TEM, Hitachi, Japan) was used to observe the appearance and size of quantum dots. The ZF-5 Portable UV analyzer was available to observe the fluorescence intensity change of the reaction system under open-air condition (Jiapeng Corporation, Shanghai, China). The pH values of the aqueous solutions were measured though utilizing a PHS-3C pH meter (Leici, Shanghai, China).

2.2. Materials and reagents

In present study, the main chemical reagents adopted were $CdCl_2{\cdot}2.5H_2O$ (Shanghai Chemicals Reagent Co., Shanghai, China), tellurium (Te) powder (Sinopharm Chemical Reagent Co., Shanghai, China), $NaBH_4$ (Tianjin Huanwei Fine Chemical Co., Tianjin, China). Glutathione (GSH) and all of the amino acids used in this paper were purchased from Aladdin Reagent Co. (Shanghai, China). Herring sperm DNA (hsDNA), yeast ribonucleic acid (RNA), bovine serum albumin (BSA), glucose, urea and collagen were obtained from Sigma (St. Louis, MO, USA). The solutions were buffered with Tris–HCl buffer solution, which was prepared by dissolving 0.1 M HCl and 0.1 M tromethamine (Tris) solution together in certain percentage. Unless stated, all reagents used were of analytical grade without further purification. Deionized distilled water prepared from a water purification machine was used throughout.

2.3. Synthesis of GSH-capped CdTe QDs

Aqueous colloids of GSH-capped CdTe QDs solution were prepared according to the previously described methods [17]. Under Ar atmosphere and magnetic stirring, Te powder (0.0383 g) was reacted with excessive sodium borohydride in deionized water to produce the colorless solution of sodium hydrogen telluride (NaHTe). $CdCl_2{\cdot}2.5H_2O$ (0.1028 g) and GSH (0.1844 g) were dissolved in 150 mL deionized water. With the slow Ar flow and vigorous stirring, the pH of the mixture was adjusted to 10.5 by using the dropwise addition of NaOH solution (1.0 M). The solution was

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