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A dual-fluorescence biosensor assembled by quantum dots and phenazinium dyes: A comparative study for DNA detection

Linlin Wang, Shaopu Liu, Chenxia Hao, Xiaolong Zhang, Chaoqun Wang, Youqiu He *

School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

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ABSTRACT

A dual-fluorescence "turn off–on" biosensor, which consists of quantum dots (GSH-CdTe QDs) whose fluorescence was quenched by safranine T (ST) via an electron transfer process, had been developed for herring sperm DNA (hsDNA) detection. Initially, in the "turn off" stage, the strong fluorescence of GSH-CdTe QDs could be effectively quenched by ST owing to the occurrence of the electron transfer from the photoexcited GSH-CdTe QDs to ST. And then, the high affinity of DNA to ST enabled the ST attached to the surface of GSH-CdTe QDs, to become embedded into hsDNA double helix structure to form stable complex and moved away from the QDs. Therefore, the recognition of hsDNA could be realized via the fluorescence restoration of the QDs–ST based biosensor, namely of the fluorescence "turn on" procedure. This designed biosensor exhibited good sensitivity and selectivity, for the reason that the detection limit for DNA reached 10.8 ng/mL, meanwhile, neither biologically relevant metal ions, common organic compounds, nor the amino acid had any significant interference in the detection mode. Hence, this simple, fast, sensitive, and selective biosensor owned perfect analysis applications in biochemical DNA monitoring.

1. Introduction

In recent years, more and more researchers pay attention to semiconductor nanoparticles which are also known as quantum dots (QDs) in recent years. QDs hold promise as fluorescent biosensors, probes, and markers for widely applications in many fields, as a result of the unique functional and structural characteristics of QDs which include small size, large absorption cross sections, narrow and Gaussian emission spectra, good stability with respect to photo-bleaching, and so on [1,2]. Earlier studies relate to the interactions of QDs with some small molecules or macromolecules, like deoxyribonucleic acids and peptides with potential medical value provide an excellent background for these phenomena by a comprehensive QDs-based fluorescence spectral analysis [3–5].

Phenazine dyes safranine T (ST, 3,7-diamino-2,8-dimethyl-5-phenyl-phenol chloride hydrochloride), neutral red (NR, 3-amino-7-dimethylamino-2-methyl phenazine hydrochloride) and phenosafranine (PS, 3,7-diamino-5-phenyl phenazinium chloride) are the most important cationic dyes which are all red coloured dyes with a planar tricyclic phenazinium moiety (Fig. 1). All the dyes have been used extensively in dye industry and have potential applications in textile, pharmaceutical, and cosmetic industries because of their brilliant staining capacity. The above dyes act as useful molecular adhesives for protein immobilization and for analyzing membrane organization and heterogeneity, as well as for the microdetection of DNA [6–10].

DNA with their evenly stacked base pairs and shallow (minor) and deep (major) grooves are attractive targets for some drug molecules. The design of effective method for DNA detection and the interactions study of drugs and DNA have been in focus, as they may reveal reaction mechanisms that may be useful for developing new specific DNA-targeted drugs. In the present investigation results on the detection of trace amounts of DNA are reported using several spectroscopic techniques, such as fluorescence [11], chemiluminescence [12], UV–vis spectroscopic measurements [13], and so on. The fluorescence based analytical method stands out from the rest for its operational characteristics, high sensitivity, and selectivity.

As we all known that a variety of factors rather than analytes can induce the ultimate fluorescence "off" state of the QDs. If the addition of analyte can remove the quencher away from the surface of the QDs, the fluorescence of QDs can recovery what is due to the specific interactions between the ligand and the analyte. As a consequence of this, the turn-on procedure can greatly reduce false

* Corresponding author.
E-mail address: heyq@swu.edu.cn (Y. He).

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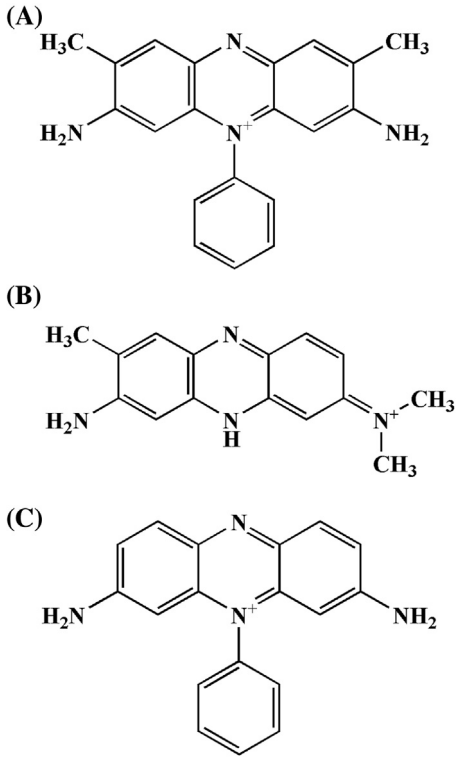
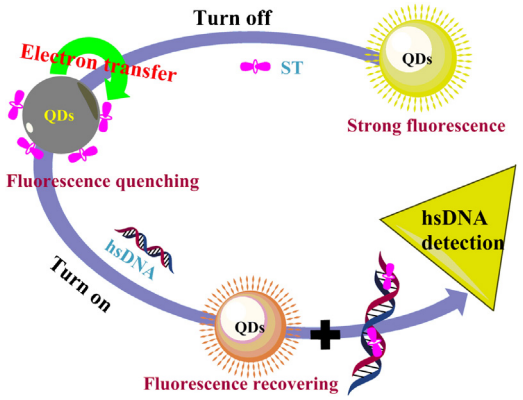


Fig. 1. Chemical structure of ST, NR, and PS.

positive results and these methods are more reliable and preferable. Hence, the "turn off–on" switch systems have drawn intensive attention for investigation and been successfully used in different biomedical labeling and detection [14]. J. Du et al. described the comparison of turn-on and turn-off detection systems of a fluorescent sensor [15]. Liang's group had synthesized the Glyp-functionalized-CdTe/CdS QDs as a turn-off fluorescence biosensor for DNA determination [16]. And Zhao et al. reported an ionic conjugate between CdTe QDs and $Ru(bpy)_2(dppx)^{2+}$ could be utilized as a fluorescent probe for the detection of DNA [17].

In this work, the interactions of phenazine dyes (like ST, NR and PS) with GSH-CdTe QDs and hsDNA was firstly reported and had been investigated in detail by means of fluorometry, Gauss View 5.08, UV–vis absorption and Resonance Rayleigh Scattering. A reversible turn "off–on" fluorescence biosensor was developed for hsDNA detection. In the meantime, Gauss View 5.08′ first using on describing the molecular structure differentia among ST, NR and PS offered a more detailed 3D image as the supporting points and made the thesis in discussion part more convincing. In despite of corresponding results revealed these phenazine dyes all could quench the fluorescence of the QDs, the fluorescence recovery induced by hsDNA was just observed in ST-modulated GSH-CdTe QDs system. Hence we realized a new fluorescent biosensor for hsDNA detection on the basis of the reversible "off–on" fluorescence change of GSH-CdTe QDs. On one hand, ST acted as the quencher to efficiently quench the high fluorescence of GSH-CdTe QDs through the electron transfer mechanism, causing the system into "off" state. On the other hand, the high affinity of ST to hsDNA enabled ST to intercalate into the hsDNA double helix structure and peeled off from the surface of GSH-CdTe QDs, recovering the fluorescence of the QDs and leading the system into "on" condition. This strategy showed good sensitivity and selectivity and avoided tedious operation, so that the comparatively accurate hsDNA detection result could be obtained (Scheme 1).



Scheme 1. Schematic diagram of the GSH-CdTe QDs–ST system based fluorescent "turn off–on" biosensor for hsDNA detection.

2. Materials and methods

2.1. Apparatus

The fluorescence with the excitation wavelength of 350 nm and the scattering intensities were both measured by a Hitachi F-2500 fluorospectrophotometer (Tokyo, Japan). The UV–vis absorption spectra were recorded by the application of a UV-2450 spectrophotometer (Tianmei Corporation, Shanghai, China). The appearance and size of quantum dots were observed though utilizing JEOL JEM-2100 transmission electron microscopy (TEM, Hitachi, Japan). The different pH values of the aqueous solutions were measured through the use of a PHS-3C pH meter (Leici, Shanghai, China).

2.2. Chemicals

The main adopted chemical reagents, cadmium chloride hemi (pentahydrate), namely $CdCl_2{\cdot}2.5H_2O$, tellurium (Te) powder, and dyes were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Sodium borohydride ($NaBH_4$) bought from Tianjin Huanwei Fine Chemical Co. (Tianjin, China). The glutathione (GSH) and all of the amino acids used in this experiment were purchased from Aladdin Reagent Co. (Shanghai, China). Herring sperm DNA (hsDNA), yeast ribonucleic acid (RNA), bovine serum albumin (BSA), glucose, sucrose, cytidine, and uridine were obtained from Sigma (St. Louis, MO, USA). The prepared Tris–HCl buffer solution was obtained by dissolving 0.1 mol/L HCl and 0.1 mol/L tromethamine (Tris) solution together in certain percentage. Ultrapure water (18.2 MΩ cm) water prepared from a water purification machine was used throughout. All reagents used were of analytical grade with no further purification.

2.3. Synthesis of GSH-CdTe QDs

GSH-CdTe QDs stabilized by GSH were prepared according to the previously described methods [18]. 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. Meanwhile, the pH of the mixture was adjusted to 10.5 through the dropwise addition of NaOH solution (1.0 mol/L). H_2SO_4 (0.5 mol/L) was introduced to the solution of NaHTe under stirring and then the generated H_2Te gas was passed through the oxygen-free Cd^{2+} solution together with the slow Ar flow. The resulting solution mixture was subjected to reflux at 369 K for one hour under open-air condition with condenser. Eventually, the salmon pink GSH-CdTe QDs solution was obtained. The concentra-