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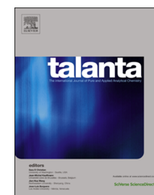

A “Turn-on-off-on” fluorescence switch based on quantum dots and gold nanoparticles for discriminative detection of ovotransferrin

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ABSTRACT

A novel strategy for ovotransferrin (OVT) detection by tracing the “on-off-on” fluorescence signals of quantum dots (QDs) and gold nanoparticles (AuNPs) utilizing fluorescence measurements were established in this article. The immune interaction between QDs-OVT and anti-OVT-AuNPs leads to drastic quenching (turning off) of the donor by a fluorescence resonance energy transfer (FRET) process. After the addition of free OVT, anti-OVT-AuNPs peel off from the QDs-OVT surface and bind to free OVT due to competitive immunoreactions, resulting in the restoration of the fluorescence intensity of QDs (turning on). Consequently, this process can be utilized for the selective detection of OVT *via* optical responses. Under optimal conditions, the fluorescence intensity of the system increased linearly with increasing concentrations of OVT from 0.05 to 3.8 μg/mL. The limit of detection of OVT was 23.55 ng/mL. This method was applied to the analysis of OVT in egg powder samples. Furthermore, the strategy provided in this work could be conveniently followed to establish similar immunosensors for the rapid detection of other proteins with corresponding antibodies.

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1. Introduction

Ovotransferrin (OVT) is an important protein in albumen, constituting approximately 12% of the total egg white protein. OVT is a branch of the bilobal glycoproteins family that tightly binds Fe^{3+}, which underlies its important function as a Fe^{3+} transport protein and helps explain the defensive role that it plays. By binding free Fe^{3+}, an essential nutrient for bacterial growth [1], OVT limits infection by both gram-positive and gram-negative bacteria [2,3]. The protein also exhibits antiviral [4,5] and antifungal activities [6]. Moreover, OVT has an immunomodulatory role [7]. OVT is similar to lactoferrin in milk in structure and biological function. It can be added to food and used as a health factor. OVT can be hydrolyzed into peptides with antibacterial activity, which can be used to improve infant resistance to intestinal bacteria. OVT has broad application prospects in medicine, drugs, and food.

Until now, the study of OVT mostly focused on the mechanism, surface modifications, and bacteriostatic activity [8]. Quantitative research on OVT is sparse, and the methods are obsolete. The earliest method of OVT detection was an enzyme-linked immunoadsordent assay, which could be used in qualitative and quantitative analyses of OVT. However, this method had a higher detection limit and involved complex operation [9,10]. The effort towards OVT analysis also focused on ultraviolet spectroscopy methods based on the characteristic absorption at 465 nm after combination with iron. The concentration of OVT could be roughly measured according to the absorption intensity [11,12]. The defect of this approach was that it could not provide accurate quantitation. A high-performance capillary isoelectric focusing technique was also used in the study of OVT. This method could analyze the degree of combination of OVT and iron ions [13]. However, it could not quantitatively detect OVT and involved complex operation and equipment. Therefore, a rapid, simple, and sensitive OVT detection method is still needed.

Advances in nanoscience and nanotechnology have resulted in various novel nanosensors, which have been conditioned for biomolecule detection both *in vitro* and *in vivo*. Among the various detection techniques, optical detection has proven to be the most convenient method of all. Fluorescence resonance energy transfer (FRET) systems possess high sensitivity and selectivity. It is a powerful technique for probing small changes in the distance between donor and acceptor fluorophores. Optimal FRET occurs when there was appreciable spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor. One of the necessary condition for FRET is that the distance between donor and acceptor is less than 10 nm. With FRET technology, the fluorescence switch can be more sensitive [14,15].

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Quantum dots (QDs) have been favorably adopted in fluorescence switch-based studies because of the large Stokes shift, high quantum yield, good photostability, and size-dependent maximum emission wavelength tunability [16,17]. The emission spectrum of QDs can be adjusted by controlling the synthesis conditions of QDs in the range wavelength of the absorption spectrum of the acceptor. With these good optical characteristics, QDs have been favorably adopted in FRET-based studies for biological analyses and applications such as cyanide ion detection [18], sulfide ion detection [19], and the treatment of water pollution [20]. Fluorescence switching perfectly combines QDs and Au nanoparticles (AuNPs). As an excellent fluorescent quencher, AuNPs open new perspectives to detect biomolecules with high sensitivity in FRET systems owing to their high extinction coefficients as well as broad absorption spectrum within the visible light range that overlaps with the emission wavelengths of common energy donors [21,22]. Thus, functionalized QDs and AuNPs were used to establish fluorescence switch.

In this study, we investigated the applicability of a QDs–AuNPs nanohybrid for an OVT sensor utilizing spectroscopic and FRET techniques. Two portions of this immunosensor, namely, QDs-OVT and anti-OVT-AuNPs, are first synthesized. As a light-absorbing material, CdTe QDs provide a "turn on" state in fluorescence measurement. This bright fluorescence is quenched in the presence of AuNPs ("turn off" state). After the addition of OVT to the QDs-OVT-anti-OVT-AuNPs system, anti-OVT-AuNPs becomes detached from the QDs-OVT surface and attached to free OVT because of competitive immunoreactions, creating a "turn on" state. The whole assay process does not require multiple time-consuming incubation, separation, and washing steps and can be rapidly accomplished within a very short time. These "turn-on-off-on" studies will create new opportunities for engineering optically based advance switches for sensing biomolecules.

2. Experiment

2.1. Reagents and chemicals

All chemicals used were analytical grade. Ovotransferrin and avidin were purchased from Sigma-Aldrich (USA). Anti-OVT was purchased from Sino-American Biotechnology Co., Ltd. (Wuhan, China). $HAuCl_4$ was purchased from the Shanghai Chemical Reagent Company (Shanghai, China). NaOH, salts (Na^+, K^+), glucose, lactose, sodium citrate, Na_2HPO_4, NaH_2PO_4, K_2CO_3, $CdCl_2$, $NaBH_4$, thioglycolic acid (TGA), N-hydroxysuccinimide (NHS), and vitamin C were acquired from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycine, cysteine, glutathione, aspartic acid, *L*-serine, tyrosine, lysozyme, and BSA were purchased from the Biosharp Company (China). PEG20000 was acquired from Beijing Solarbio Science and Technology Company (China). HCl was acquired from the Xinyang Chemical Reagent Factory (China). Te powder was acquired from Delan Fine Chemical Reagent Company (Tianjin, China). 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 1-Thioglycerol (TG) were acquired from Aladdin Chemical Reagent Company Ltd. (China). Albumin was purified in our lab. Doubly distilled water (DDW) was used throughout.

OVT was prepared in a 5 mg/mL stock solution and diluted to 0.01 mg/mL as the working solution. Anti-OVT was prepared at 10 μg/mL. All the solutions were stored at 4 °C. The 0.1 mg/mL $HAuCl_4$ and 10 mg/mL sodium citrate solutions were prepared with DDW. Phosphate buffered solutions (PBS) with different pH values were prepared by mixing 0.01 mol/L Na_2HPO_4 and 0.01 mol/L NaH_2PO_4, according to certain proportions.

Egg products (egg white powder, yolk powder, and whole egg powder) were purchased from the Kangde Biological Products Co. Ltd. (China).

2.2. Preparation and characterization of the AuNP solution

The glass apparatus used for preparing AuNPs was immersed in aqua regia (HNO_3:HCl = 1:3, v/v) for 48 h, washed by DDW several times, and then dried before use.

AuNPs were prepared according to previously published methods [23]. In a 250 mL beaker, 100 mL of 0.1 mg/mL $HAuCl_4$ was heated at 95 °C until boiling. Next, 5 mL of 10 mg/mL sodium citrate solution with the same temperature was quickly added to the beaker drop by drop and the solution was stirred at 120 rpm on a magnetic heater stirrer (SZCL-2A magnetic stirring apparatus from Shanghai Dongxi Refrigeration Equipment CO., Ltd, China). AuNPs formed in 2–3 min [24]. The solution was boiled with stirring for 10 min. The color of the solution turned from blue-black to bright red. The cooled solution was stored at 4 °C, sealed and in the dark. The concentration of the above AuNP solution was 47.8 μg/mL.

A JEM-2100 transmission electron microscope (TEM) (JEOL Ltd, Japan) was used to observe the formation of AuNPs and to measure their diameters. AuNPs were diluted to suitable concentrations and ultrasonically dispersed for 10 min. One to two drops were placed on a copper network and the copper network was placed on the rod sample for TEM detection after water volatilization. The acceleration voltage was 200 KV.

2.3. Conjugation of AuNPs with anti-OVT

To prepare the anti-OVT-AuNPs nanoprobe, 0.075 mL of 10 μg/mL anti-OVT was added to 4 mL of 47.8 μg/mL AuNPs with the appropriate pH with magnetic stirring [25]. After stirring for 10 min, 20 μL of 3% PEG20000 was added as a stabilizer. PEG20000 acted as a dispersing agent in the combination of anti-OVT and AuNPs. It can prevent the spontaneous aggregation of the anti-OVT-AuNPs. Next, the mixture was stirred for 15 min and stored at 4 °C. The conjugation process was optimized by altering the conjugation pH value.

The combination of AuNPs and anti-OVT can be controlled by adjusting the pH in relation to the electrostatic interaction. The pH values of the AuNPs were adjusted using 0.1 mol/L K_2CO_3 or 0.1 mol/L HCl (PHS-3C pH meter from Shanghai Precision & Scientific Instrument CO., Ltd, China). In a 10 mL tube, 0.5 mL of 47.8 μg/mL AuNPs with different pH values and 0.075 mL of 1.25 μg/mL anti-OVT were added. After 5 min, 50 μL of 10% KCl was added. The solution was diluted with DDW to 5 mL and mixed thoroughly after 30 min. The resonance Rayleigh scattering (RRS) intensity of the mixed solution was detected using RF-5301 Spectrofluorometer (Shimadzu, Japan). The RRS intensity of the solution were recorded by means of synchronous scanning at $\Delta\lambda = 0$ ($\lambda_{em} = \lambda_{ex}$) through wavelengths ranging from 225 to 800 nm. The excitation and emission slit width were 5.0 nm. The RRS intensity of the binding product and the blank were measured at the maximum wavelength.

2.4. Synthesis of CdTe QDs and conjugation of CdTe QDs with OVT

Referring to the existing method [26,27], highly fluorescent CdTe QDs were synthesized in aqueous solution. The oxygen-free NaHTe solutions were prepared by stirring a mixture of 36.4 mg of $NaBH_4$, 26.7 mg of Te powder, and 1.5 mL of DDW at 37 °C until the black Te powder disappeared (AR-2140 analytical balance from Mettler Toledo instrument CO., Ltd, Switzerland). The freshly prepared oxygen-free NaHTe solution reacted with a mixture of 160 mL of 0.0026 mol/L $CdCl_2$, 140 μL of TG, and 27.5 μL of TGA

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