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Immunoassay detection using functionalized gold nanoparticle probes coupled with resonance Rayleigh scattering

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ABSTRACT

Functionalized gold nanoparticle probes coupling with resonance Rayleigh scattering (RRS) has been developed for the picomolar detection of transferrin. This assay relies on the specific immune recognition of nanoprobe that is modified with anti-transferrin antibody on gold nanoparticle surface through cysteamine bioconjugation. Transmission electron microscopy (TEM), laser light scattering and UV–vis absorption spectroscopy were employed to construct and characterize the nanostructures and spectroscopic characteristics of nanoprobes. Furthermore, the experiment conditions, such as nanoprobe concentration, reaction temperature, incubation time, pH value, salt concentration and coexisting substances, are optimized. The combination of signal amplification of nanoprobes with high sensitivity of RRS technique allow the detection limit of transferrin to reach 85 pM, and the linear detection range from 85 pM to 3.4 nM. This method has been successfully applied to detect transferrin of human serum samples with good reproducibility, and it has the potential of being widely used in clinical diagnosis.

1. Introduction

Highly sensitive detection and accurate analysis of specific target molecules in human serum samples are important for early clinical diagnosis and treatment. Significant efforts have been made in recent years to apply nanoprobes to realize highly sensitive bioassay, such as the detection of DNA sequence [1,2], antibody [3], and antigen [4,5]. Nanoprobes initially are fabricated by a sequential self-assembling of functional molecules and specific antibodies on nanoparticle surface. After the recognition of nanoprobes with specific target from the sample solution, the target molecule concentration can then be determined. Compared with conventional immunoprobes, nanoprobes have some functional features such as signal amplification of nanoparticles and high capacity to form immune complex. However, the acquisition of highly sensitive detection signal is a challenging task in the area of trace detection.

Gold nanoparticles are known to have large light absorption and scattering cross section in the surface plasmon resonance wavelength regions [6,7]. The magnitude of light scattering by gold nanoparticles can be orders of magnitude higher than light emission from fluorescent dyes. These unique properties have many important and promising applications in the biomedical field, such as molecular imaging, biosensing, and bioassays [8–10]. Although the strong light scattering property of gold nanoparticles has been mainly used in optical microscopic imaging of biological cells, the application in quantitative analysis and assay is rather limited.

Resonance Rayleigh scattering (RRS), a special re-scattering, takes place when the wavelength of Rayleigh scattering is located at or close to the molecular absorption band [11]. Therefore, compared with other spectroscopic techniques, RRS detection limit is lowered by several orders of magnitude. RRS technique has been used to determine macromolecules, such as inorganic ions, organic compounds, nucleic acid, and protein [12–16]. Because of the unique RRS property of gold nanoparticles, it is natural to hypothesize that RRS can be a very sensitive technique for quantitative detection and analysis of nanoprobes at low concentration. Indeed, it has been reported that the detection of human serum albumin is possible through nanoparticles aggregation-based RRS, whereas the detection limit only reaches nanomolar level [17]. Although extensive studies have been reported on bioconjugation of gold nanoparticles and immune recognition of nanoprobes [3,18], to our knowledge, RRS has not been used in conjunction with antibody-modified nanoprobes for quantitative immunoassay. RRS can distinguish individual nanoprobe with immune recognition-induced immunocomplex because of the difference between their particle sizes, and this capability makes RRS as a potential analytical tool for a quantitative immunoassay.

Human holotransferrin (TF), a type of iron-binding proteins, is the principal iron transport of circulating blood [19]. It is a clini-

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cal biomarker for protein-calorie malnutrition [20] and a potential marker for diabetes [21]. In human serum, the total TF concentration is in the range of 2–4 mg/ml (25–50 μM) [22]. Studies have shown that the serum level of TF is lower in patients with early malnutrition [23], whereas higher in patients with diabetes [24] than the healthy people.

Although enzyme-linked immunosorbent assay (ELISA) [25] and electrode-based immunosensor [26,27] have been used in TF detection, their detection limits only reach nanomolar level and these methods have obvious drawbacks, such as time-consuming and poor reproducibility, respectively. It is therefore essential to achieve the lower detection limit by combining highly sensitive RRS technique with signal amplification of nanoprobes.

We herein report the development of a highly sensitive immunoassay for antigen detection using functionalized nanoprobes coupled with RRS technique. Transmission electron microscopy (TEM), laser light scattering, UV–vis absorption and RRS spectra are used to characterize the nanostructures, bioconjugation and RRS characteristics of fabricating nanoprobes that are immobilized by a sequential self-assembling of cysteamine and anti-TF antibody on gold nanoparticle surface. We then use this anti-TF-conjugated nanoprobe as a prototype to elucidate a picomolar detection limit for the specific TF. Furthermore, we analyze the selectivity of anti-TF-conjugated nanoprobes and optimize the experiment conditions.

2. Experimental

2.1. Chemicals

Human holotransferrin (TF), bovine serum albumin (BSA), lysozyme, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), cysteamine were purchased from Sigma (St. Louis, Mo, USA). Goat monoclonal anti-human transferrin antibody (anti-TF) was obtained from Shanghai Biochemical Reagents Company (Shanghai, China). Anti-TF antibody was diluted at a working titer of 1:40 in 0.1 M phosphate-buffered saline (PBS) with pH 7.4. Trisodium citrate and chloroauric acid were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). 0.1 M PBS (pH 7.4) was used in all experiments. All reagents used in the experiments were analytical grade, and all solutions were prepared in high purity water (Milli-Q, Millipore).

2.2. Apparatus and methods

2.2.1. UV–vis absorption measurements

Absorption spectra were recorded by a UV-1901 UV–vis spectrophotometer (Beijing, China) using quartz cell with the path length of 1.0 cm.

2.2.2. Dynamic light scattering measurements

Size distribution of various nanoparticles was measured by laser light scattering following their resuspension in 0.1 M PBS (pH 7.4) using a using a Zetasizer Nano-ZS (Malvern Instruments, UK).

2.2.3. Resonance Rayleigh scattering measurements

Resonance Rayleigh scattering (RRS) measurements were performed with a 970CRT fluorescence spectrophotometer (Shanghai, China) with a 1.0 cm quartz cell. The magnitude of RRS intensity was obtained by the synchronous scanning at $\lambda_{ex} = \lambda_{em}$ ($\Delta\lambda = 0$ nm). The relative RRS intensity (ΔI_{RRS}) was obtained by the difference between the assay system (I_{RRS}) and the regent blank (I_0), namely, $\Delta I_{RRS} = I_{RRS} - I_0$.

2.2.4. Transmission electron microscopy (TEM) images

TEM images of nanoparticles were characterized by a Philips TECNAI-10 transmission electron microscope. Samples were prepared by dropping onto copper grid.

2.3. Synthesis of gold nanoparticles

Gold nanoparticles (GNP) were prepared by the reduction of chloroauric acid with trisodium citrate. Briefly, 10 ml of 1% trisodium citrate was quickly added to 50 ml of the boiling chloroauric acid solution (1.0×10^{-3} M) under vigorous stirring. The color of the solution turned from pale yellow to wine red. This solution was allowed to react while stirring under heating for 15 min. When the heating mantle was removed, this solution was stirred under room temperature for another 2 h.

2.4. Fabrication of anti-TF-conjugated nanoprobes

1 ml of GNP was mixed with 200 μl of 0.2 M deoxygenated cysteamine solution for 12 h at 4 °C. Cysteamine-modified GNP was separated by centrifugation (5000 rpm, 10 min) and washed with PBS (pH 7.4) three times to remove unimmobilized cysteamine. The amine-functional GNP was re-dispersed in 1 ml of PBS (pH 7.4). In order to conjugate anti-TF antibody with GNP surface, 80 μl anti-TF was incubated with 1 ml of cysteamine-modified GNP solution containing 0.25 M EDC for 12 h at 4 °C. Then antibody-conjugated GNP solution was separated by centrifugation (5000 rpm, 10 min) and washed three times with PBS (pH 7.4) to remove unconjugated anti-TF. Finally, antibody-conjugated nanoprobes were re-dispersed in 1 ml of PBS (pH 7.4) and kept at 4 °C.

2.5. Preparation of human serum samples

Human serum samples were from healthy adult volunteers. Blood samples were separated by centrifugation (1500 rpm, 10 min) to remove erythrocytes and kept at 4 °C.

3. Results and discussion

3.1. Characterization of anti-TF-conjugated nanoprobes

One of the important steps for developing a useful nanoprobe is to fabricate functionalized nanoparticle that can facilitate the conjugation of antibody with immune activity. Self-assembly strategy has proven to be a well-developed technique for constructing a highly functional interface, which can provide an excellent surface to realize antibody immobilization [28,29]. We therefore fabricate antibody-conjugated nanoprobe through modifying GNP with cysteamine and conjugating the amine-functional surface with anti-TF antibody (Scheme 1). Dynamic laser light scattering is applied to measure particle sizes of GNP and antibody-conjugated nanoprobe, whereas TEM is used to visually characterize their nanostructures, respectively. Measurements with dynamic laser light scattering showed that these nanoparticles had average diameter of 20 nm for GNP alone and 50 nm for anti-TF-labeled nanoprobe, indicating that the coupling of antibody to GNP occurred. Under TEM, individual GNP exhibited spherical and well-shared shape (Fig. 1A). Conjugation of anti-TF antibody onto GNP surface induced the increase in average diameter of nanoparticle, and the edge of antibody-conjugated nanoprobe has white halo disturbance (Fig. 1B), visually demonstrating that anti-TF is covalently attached on GNP surface through cysteamine layer linkage.

Another evidence of antibody linkage on GNP surface can be provided by UV–vis absorption spectra. GNP exhibited the absorp-