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Determination of human IgG by solid substrate room temperature phosphorescence immunoassay based on an antibody labeled with nanoparticles containing dibromofluorescein luminescent molecules

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Abstract

Luminescent silicon dioxide nano-particles with size of 20 nm, which containing dibromofluorescein (D) were synthesized by sol–gel method (symbolized by D–SiO_2).The particles can emit intense and stable room temperature phosphorescence signal on polyamide membrane when $Pb(Ac)_2$ was used as a heavy atom perturber. The $\lambda_{exmax}/\lambda_{emmax}$ was 457/622 nm. Our research indicated that the specific immune reaction between goat-anti-human IgG antibody labeled with D–SiO_2 and human IgG could be carried out on polyamide membrane quantitatively, and the phosphorescence intensity of the particle was enhanced after the immunoreactions. Thus a new method of solid substrate room temperature phosphorescence immunoassay (SS-RTP-IA) for the determination of human IgG was established basing on antibody labeled with the D–SiO_2 nanoparticles. The linear range of this method was 0.0624–20.0 pg human IgG spot^{-1} (corresponding concentration: 0.156–50.0 ng ml^{-1}, the sample volume: 0.40 μl spot^{-1}) with a limit of detection (LD) as 0.018 pg spot^{-1}, and the regression equation of working curve was ΔI_p = 7.201 m_{IgG} (pg spot^{-1}) + 82.57. Samples containing 0.156 and 50.0 ng ml^{-1} of IgG were measured repeatedly for 11 times and R.S.D.s were 4.1 and 3.4%, respectively. Results showed that this method had the merits as sensitive, accurate and precise.

Keywords: Luminescent nanoparticle; Solid substrate room temperature phosphorescence immunoassay; Human IgG; Dibromofluorescein

1. Introduction

At present, many different techniques have been developed to study antigen–antibody reaction as well as to provide an effective method for clinical diagnosis. But all the previous assays each have their drawbacks: Radioimmunoassay (RIA) requires radioisotopes and all the precautions and hazards of their use. Enzyme immunoassay (EIA) needs active enzymes which require special storage. The sensitivity of FIA is determined by the non-specific background signals caused by auto-fluorescence of the biological objects, fixative-induced fluorescence, and natural fluorescence of components [1]. Meanwhile, there are some methods applied to the determination of phosphorescence, such as micelle-stabilized phosphorescent immunoassay, MS-PhIA [2] and dry surface phosphorescent immunoassay, DS-PhIA [3] etc. But these methods have low sensitivity. Compared with fluorimetry, phosphorimetry has many merits such as larger Stokes' shift, easier to reduce the interference of background fluorescence and scattering light, longer lifetime and better selectivity etc. Solid substrate room temperature phosphorescence is the most sensitive method in all room temperature phosphorinmetry. It requires only lambdas of sample solution. Additionally, the phosphorescence measurement can be carried out directly after various operations in immune reaction on substrates. The simple operation is similar with that of enzyme-linked immunoassay. Thus the method combining

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solid substrate room temperature phosphorescence with immunoassay is established with the name as solid substrate room temperature phosphorescence immunoassay (SS-RTP-IA). In our previous work, fluorescein isothiocyanate (FITC) [4] and tetramethylrhodamine B isothiocyanate (TRITC) [5] have been used respectively to label goat-anti-human IgG antibody in the developed SS-RTP-IA of human IgG, and the limits of detection in the methods are 0.32 pg spot^{-1} and 0.37 pg spot^{-1} (sample volume: 0.40 μl spot^{-1}), respectively. It is well known that two important ways to increase the sensitivity of assay are enhancing the signal intensity of sample and reducing the interference of background. The improvement of sample signal in immunoassay is directly related to the density of the labeling molecules, or the number of luminescent molecules linked with each antibody (or antigen). Therefore, using nanoparticles containing multi-luminescent molecules as labeling reagent should be an efficient way to improve the sensitivity of SS-RTP-IA. Although there have been some reports about using nanoparticles containing dye molecules as fluorescent labeling reagent [6] or the sale of luminescent nanoparticle [7], it is regretful that there are no more reports concerning about using nanoparticles containing luminescent molecules as phosphorescent labeling reagent, except the discussion about the feasibility of phosphorescence analysis based on luminescent nanoparticles prepared by glutaraldehyde polymerization with FITC [8]. In this paper, the silicon dioxide nanoparticles containing dibromofluorescin (symbolized as D–SiO_2) were prepared by Sol–Gel technology, using Na_2SiO_3 as the precursor and doping it with D. Meanwhile, the room temperature phosphorescence properties of the nanoparticles and antibody labeled with it were studied. It is found that goat-anti-human IgG antibody labeled with the nanoparticle (symbolized as D–SiO_2–Ab) could react quantitatively with human IgG on polyamide membrane. After the immune reaction, not only the immune complex retained the excellent room temperature phosphorescence properties of D–SiO_2, but the phosphorescence intensity could be measured at the wavelengths of D. Based on the facts, we established a new SS-RTP-IA method for the determination of human IgG, using nanoparticles containing dibromofluorescein as the marker for antibody. The linear range of this method is 0.0624–20.0 pg human IgG spot^{-1} with a sample volume of 0.40 μl (corresponding concentration:0.156–50.0 ng ml^{-1}). Limit of detection calculated by 3Sb/k was 0.018 pg spot^{-1}, which was 17.8 times of that had been reported [4]. This method has been applied in the determination of IgG in human serum with satisfactory results.

2. Experimental

2.1. Apparatus and reagents

A Perkin-Elmer LS-55 luminescence spectrophotometer with a front-surface attachment. The instrument's main parameters are as following: delay time:0.1 ms, gate time:2.0 ms, cycle time:20 ms, flash count:1, ex. slit:10 nm, em. slit:15 nm, scan speed:1500 nm/min); AE240 Electronic analytical balance (Mettler-Toledo company); KQ-250B ultrasonic washing machine (Kunshan Ultrasonic machine company); 0.5 μl flat head micrometer syringe (Shanghai Medical Laser Instrument plant); JEM-2000EX transmission electron microscope (TEM, electric company, Japan).

Dried powder of human serum immunoglobulin (Banding Taike Bio-Tech company, stored at 0–4 °C); Goat-anti-human IgG antibody (Xiamen Univ anti-cancer research center, 21 mg ml^{-1}); Tris (hydroxymethyl) aminomethane; Tween-20; BSA (bovine serum albumin); HCl; NaCl. All reagents were in A.R grade. The water used was thrice distilled water.

Preparation of 0.1 mol l^{-1} Na_2CO_3–$NaHCO_3$ buffer solution: 200 ml of 0.1 mol l^{-1} Na_2CO_3 and 800 ml of 0.1 mol l^{-1} $NaHCO_3$ were mixed together. The pH value were 9.4 at 20 °C and 9.1 at 37 °C.

Preparation of 0.05 mol l^{-1} Tris–HCl buffer solution: 250 ml of 0.2 mol l^{-1} Tris and 400 ml 0.1 mol l^{-1} HCl solution which containing 150 mmol l^{-1} NaCl were mixed together, then the mixture was diluted to 1000 ml. The pH value was 7.4 at 25 °C.

Preparation of washing buffer solution (Tris–HCl–0.10% Tween-20): 1.00 ml of Tween-20, 250 ml of Tris solution (0.20 mol l^{-1}) and 400 ml of HCl solution (0.10 mol l^{-1}) containing 150 mmol l^{-1} NaCl were mixed together, and then diluted the mixture to 1000 ml with water.

Preparation of solution contained bovine serum albumin (BSA): BAS was diluted to 10.00 mg ml^{-1} with 0.10 mol l^{-1} Na_2CO_3–$NaHCO_3$ buffer solution and then stored at 4 °C for the following experiment.

Preparation of 1 mol l^{-1} $Pb(Ac)_2$ solution: $Pb(Ac)_2$ was dissolved by 2 mol l^{-1} HAc solution. It was used as the heavy atom pertueber.

Filter paper used was purchased from Xinhua paper Corporation (Hangzhou, China). Acetyl cellulose membrane, nitrocellulose membrane and polyamide membrane were purchased from Luqiaosijia biochemical plastic plant (Hangzhou, China). The paper sheet were precut into small wafers[(Φ) diameter = 15 mm], with a ring indentation (Φ = 4.0 mm) at the center of each wafer made by a standard pinhole plotter.

2.2. Experimental method

2.2.1. Synthesis and surface modification of D–SiO_2 nanoparticles

100.0 ml of 1.0 (g l^{-1}) Na_2SiO_3 and 5.00 ml of dibromofluorescein (1.0 × 10^{-4} mol l^{-1}) were mixed together, then add 12 mol l^{-1} hydrochloric acid dropwise into the mixture while stir at a temperature below 30 °C until the mixture turned into limpidity sol (pH = 9.0). Followed by 30 min's standing, 6.0 mol l^{-1} hydrochloric acid was added again with stirring around 90–92 °C until the pH value reached 3.0.