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Determination of trace human chorionic gonadotropin by using multiwall carbon nanotubes as phosphorescence labeling reagent

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ABSTRACT

Taking advantage of the cutting effect of the strong oxidation of benzoyl peroxide [$(C_6H_5CO)_2O_2$] on the end of multiwall carbon nanotubes (MWNTs) to obtain water-soluble multiwall nanotubes (MWNTs') and the spiking effect of polyacrylamide (PA) on the room temperature phosphorescence (RTP) of MWNTs', a new phosphorescent labeling reagent, MWNTs'–PA, has been developed in this study. The product β-Ab_{HCG}–MWNTs'–PA obtained by MWNTs'–PA labeling human chorionic gonadotropin-β-subunit three-dimensional core monoclonal antibody (β-Ab_{HCG}) not only could maintain good RTP characteristics of MWNTs' but also could take specific immunoreaction with β-HCG to form β-HCG–β-Ab_{HCG}–MWNTs'–PA, resulting in the increase of MWNTs' RTP signal. Thus, a new solid substrate room temperature phosphorescence immunoassay (SSRTPIA) for the determination of β-HCG has been established. The limits of detection (LODs) of the new method were 0.021 pg $spot^{-1}$ for the direct way at 447/615 nm ($\lambda_{ex}^{max}/\lambda_{em}^{max}$) and 0.016 pg $spot^{-1}$ for the sandwich way at 447/614 nm ($\lambda_{ex}^{max}/\lambda_{em}^{max}$). This sensitive, accurate, and precise method was used to determine β-HCG and diagnose human diseases by the direct way or the sandwich way, with the results coinciding with those obtained by chemiluminescence immunoassay. Meanwhile, the mechanisms of MWNTs' labeling β-Ab_{HCG} and determining β-HCG are also discussed.

Human chorionic gonadotropin (β-HCG)[1] has been applied widely in the prediction of pregnancy-induced hypertension syndrome, reminding abnormal pregnancy (e.g., threatened abortion, fallopian tube pregnancy), progestation screening for Down's syndrome, diagnosis and monitor of pregnancy trophoblastic disease (e.g., acephalocystis racemosa, chorioepithelioma), treatment of threatened abortion, habitual abortion, pedo-cryptorchidism and male sterility, and so forth [1–5]. Obviously, the content of β-HCG is closely related to human diseases and has important meaning for clinical detection and diagnosis.

β-HCG, consisting of α-subunits, β-subunits, and 237 amino acids, exists in blood and urine of pregnant women. The α-subunit of β-HCG is similar to that of follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone, which can carry out immune cross-reaction, whereas the β-subunit of β-HCG is a specific chain, which can be detected by its immunoreaction with β-subunit three-dimensional core monoclonal antibody (β-Ab_{HCG}) [6]. Many methods for the determination of β-HCG have been reported based on the specificity of the immunoreaction between β-HCG and β-Ab_{HCG} such as resonance scattering spectral assay (RSSA) [7], radioimmunoassay [8,9], chemiluminescence immunoassay [10,11], fluorescence immunoassay [12,13], electrochemical immunoassay [14–17], and colloidal gold-labeled spectrophotometry [18].

However, these methods have some limitations. The selectivity of RSSA was poor due to its insufficient antiinterference ability; radioimmunoassay has great diversity as a result of different standards used in different laboratories [19] and other demerits such as poor reagent stability, short period of validity, complex operation,

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[1] *Abbreviations used:* β-HCG, human chorionic gonadotropin; β-Ab_{HCG}, human chorionic gonadotropin-β-subunit three-dimensional core monoclonal antibody; RSSA, resonance scattering spectral assay; SSRTPIA, solid substrate room temperature phosphorescence immunoassay; SSRTP, solid substrate room temperature phosphorescence; IgG, immunoglobulin G; MWNT, multiwall nanotube; MWNTs', water-soluble MWNTs; multiwall nanotube PA, polyacrylamide; BSA, bovine serum albumin; PBS, phosphate buffer solution; EDC, 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide; ACM, acetate cellulose membrane; NCM, nitrocellulose membrane; PAM, polyamide membrane; RTP, room temperature phosphorescence; LH, luteotropic hormone; FSH, follicle-stimulating hormone; TSH, thyrotropic-stimulating hormone; SEM, scanning electron microscope; RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification; CLIA, chemiluminescent immunoassay; AP, alkaline phosphatase; IR, infrared.

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and radioactive contamination [20]; spectrophotometry has low sensitivity and easily appears in false negative results; chemiluminescence immunoassay needs a long incubation time; regeneration of capillary in fluorescence immunoassay is time-consuming and easily influences the activity of antibody; and electrochemical immunoassay has a low signal-to-noise ratio. Therefore, to find a new highly sensitive, accurate, and rapid method for the determination of β-HCG is of high academic value and significance; at the same time, this method has wide application for the early warning and prevention of major diseases in humans.

Solid substrate room temperature phosphorescence immunoassay (SSRTPIA), established by combining the high sensitivity of solid substrate room temperature phosphorescence (SSRTP) with the high specificity of biological reaction, needs only microliter samples and can be easily conducted on many kinds of substrates. The sample operations, suspending samples, washing, drying, and measurement of phosphorescence, are similar to those of enzyme-linked immunoassay (ELISA) showing the bright development foreground of SSRTPIA [21].

The development of SSRTPIA largely depends on the exploitation of phosphorescent labeling reagents. Recently, eosin–isothiocyanate molecules [22–24], luminescent nanoparticles silicon dioxide containing fluorescein isothiocyanate molecules [25,26], rhodamine 6G molecules [27,28], dibromofluorescein molecules [29,30], and rhodamine 6G-dibromofluorescein molecules [31] have been successfully used as phosphorescence labeling reagents for the determination of complement 3 [22–24], immunoglobulin G (IgG) [25–30], and α-fetoprotein variant (AFP-V) [31] in human serum. Because of the excellent mechanical and electrochemical performances of multiwall nanotubes (MWNTs), they have become an advancing subject in the material science research field [32,33]. Research shows that when MWNTs are fractured, the carbon atoms of the fracture have high activity and can be easily oxidized to the –COOH group [34]. Reagents used to introduce –COOH functional groups on MWNTs include concentrated HNO_3 [35], H_2SO_4–HNO_3, and H_2SO_4–H_2O_2. Therefore, MWNTs treated with H_2SO_4–H_2O_2 have been used for the determination of trace lead [36].

In this study, we successfully determined β-HCG in human serum and predicted human diseases with SSRTPIA based on the specific immunoreaction between β-HCG and β-Ab_{HCG}–MWNTs' (water-soluble MWNTs)–PA (polyacrylamide). To the best of our knowledge, there have been only rare reports on the technology of $(C_6H_5CO)_2O_2$ cutting the end of MWNTs and the detection of β-HCG by using cutting products MWNTs'–PA to label β-Ab_{HCG}. This study not only opens up the application of MWNTs in SSRTPIA but also discusses the mechanisms of both MWNTs'–PA labeling β-Ab_{HCG} and the determination of β-HCG, which had new breakthroughs in methodology of SSRTP and immunoassay.

Materials and methods

Apparatus and reagents

Phosphorescent measurements were carried out on a PerkinElmer LS-55 luminescence spectrophotometer with a solid surface analysis apparatus (PerkinElmer). The instrument's main parameters were as follows: delay time, 0.10 ms; gate time, 2.0 ms; cycle time, 20 ms; flash count, 3.0; excitation (Ex) slit, 10 nm; emission (Em), 15 nm; scan speed, 1500 nm min^{-1}. A KQ-250B ultrasonic washing machine (Kunshan Ultrasonic Machine), an AE240 electronic analytical balance (Mettler–Toledo Instruments), and an SHP-250 biochemical incubator (Shanghai Instrument) were also used. A 0.50-μl flat head microinjector (Shanghai Medical Laser Instrument) was used to introduce the solution at a microliter (μl) level.

β-HCG, β-Ab_{HCG}, and bovine serum albumin (BSA) were purchased from Wako Pure Chemical Industries. Then, 1.0×10^{-5} g ml^{-1} β-HCG phosphate buffer solution (PBS), 1.0×10^{-5} g ml^{-1} β-Ab_{HCG} PBS solution, and 10.0 mg ml^{-1} BSA water solution were prepared as stocking solution and stored at 0 to 4 °C. 1.0×10^{-5} g ml^{-1} β-HCG was gradually diluted to 50.0, 40.0, 30.0, 20.0, 15.0, 10.0, and 1.0 ng ml^{-1} with PBS, and 1.0×10^{-5} g ml^{-1} ß-Ab_{HCG} was gradually diluted to 50.0, 25.0, 10.0, 5.0, and 1.0 ng ml^{-1} with PBS as working solution before use.

Next, 1.00 mol L^{-1} $Pb(Ac)_2$ (dissolved with 2.0 mol L^{-1} HAc), 1.0% PA water solution (MW ⩾ 3000,000), 3.0 g L^{-1} $(C_6H_5CO)_2O_2$–C_2H_5OH (anhydrous ethanol), and 0.010 mol L^{-1} PBS (5.90 g Na_2HPO_4, 0.50 g NaH_2PO_4, and 9.00 g NaCl) were weighed and dissolved with water and diluted to 1000 ml (the pH value of buffer solution was 7.4). PBS–0.05% Tween 20 washing buffer solution (5.90 g Na_2HPO_4, 0.50 g NaH_2PO_4, and 9.00 g NaCl) was weighed and dissolved with water, and then 0.50 ml of Tween 20 was added and diluted to 1000 ml. MWNTs (particle sizes: 10–20, 20–40, and 40–60 nm; Shenzhen Nanotech Port) and KBr (Sigma, contents >99.99%) were also used in the experiment.

Preparation of EDC–NHS coupling agent solution

The mixture of 5 mM 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC, Alfa) and 5 mM *N*-hydroxysuccinimide (NHS, Alfa) was prepared with 40% ethanol. Among these reagents, β-HCG, β-Ab_{HCG}, and BSA were biochemical reagents, KBr was of spectral pure, and the other reagents were of analytical (AR) grade. The water used was thrice-distilled water.

Acetate cellulose membrane (ACM), nitrocellulose membrane (NCM), and polyamide membrane (PAM) were purchased from Luqiaosijia Biochemical Plastic Plant. They were cut into wafers (diameter of 15 mm), and a ring indentation (diameter of 4.0 mm) was made at the center of each sheet with a standard pinhole plotter for use.

Experimental method

Synthesis of MWNTs'–PA labeling reagent

First, 0.30 g of $(C_6H_5CO)_2O_2$ and 10.0 ml of C_2H_5OH were added to a 50-ml Erlenmeyer flask. The flask was kept in an ultrasonic oscillator until $(C_6H_5CO)_2O_2$ was dissolved completely. Then, 0.0030 g of MWNTs was added to $(C_6H_5CO)_2O_2$–C_2H_5OH solution, ultrasonic refluxed at 80 °C for 5 h, and cooled down to room temperature before being filtered by a sand funnel with a 0.20-μm porous membrane and washed with C_2H_5OH until the filtrate was neutral. The filtrate was collected, and 0.50 ml of 1.0% PA was added to the volumetric flask and diluted to 100.0 ml with water, and then water-soluble phosphorescent labeling reagent MWNTs'–PA (2.0×10^{-6} g ml^{-1} MWNTs'–0.0050% PA) was gained. Compared with the method in Ref. [37], this simple and rapid method without H_2SO_4, HNO_3, and H_2O_2 avoided overoxidation.

Labeling of β-Ab_{HCG}

According to Ref. [38], 0.40 μl of β-Ab_{HCG} solution of different concentrations (w/v; the solution was diluted with PBS, pH 7.4) and volumes was suspended onto the center indentation of ACM with a 0.50-μl flat head microinjector. Then, ACM was taken out, on which 0.40 μl of labeling reagent (MWNTs'–PA) and EDC–NHS was dropped. The sample was kept at 37 °C for 2 h, washed three times with washing buffer solution by ultrasonic oscillator to remove unreacted labeling reagent on ACM, and removed water by filter paper. Finally, β-Ab_{HCG}–MWNTs'–PA labeling product was obtained for use. The optimal concentration and volume of β-Ab_{HCG} were chosen according to the results of immunoreaction between β-Ab_{HCG}–MWNTs'–PA and β-HCG of different