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Fullerol-fluorescein isothiocyanate-concanavalin agglutinin phosphorescent sensor for the detection of alpha-fetoprotein and forecast of human diseases

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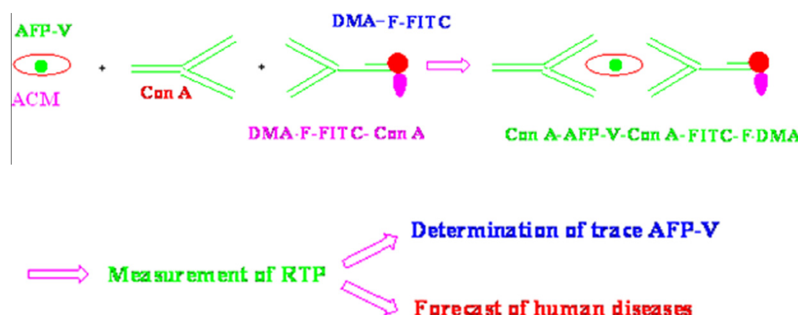
HIGHLIGHTS

- Fullerol-fluorescein isothiocyanate and N,N-dimethylaniline (F-FITC-DMA) was exploited.
- Phosphorescent sensor has been designed using reaction between F-FITC-DMA and Con A.
- The sensor could be applied to the detection of AFP-V and the forecast of diseases.
- Mechanisms of labeling Con A and the determination of AFP-V were discussed.
- The coupling technique of sensor, labeling method and phosphorimetry was developed.

GRAPHICAL ABSTRACT

A new phosphorescent sensor for the determination of alpha-fetoprotein variant (AFP-V) and the technical method for the forecast of human diseases have been developed based on the specific affinity adsorption reaction between –COOH of DMA-F-FITC-Con A and –NH_2 in AFP-V. This study opened up the analytical application of sensor, F and lectin, but also provided a new way for the development of SSRTP.



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ABSTRACT

Based on the reaction of the active –OH group in fullerol (F) with the dissociated –COOH group in fluorescein isothiocyanate (FITC) to form an F-FITC and the enhanced effect of N, N-dimethylaniline (DMA) on phosphorescence signal of F-FITC, a new phosphorescent labeling reagent (DMA-F-FITC) was developed. What's more, a phosphorescent sensor for the determination of alpha-fetoprotein variant (AFP-V) has been designed via the coupling technique of the high sensitivity for affinity adsorption–solid substrate-room temperature phosphorimetry (AA-SS-RTP) with the strong specificity reaction between DMA-F-FITC-Con A and AFP-V. The DMA-F-FITC increased the number of luminescent molecules in the biological target which improved the sensitivity of phosphorescent sensor. The proposed sensor was responsive, simple, selective and sensitive, and it has been applied to the determination of trace AFP-V in human serum and the forecast of human diseases using phosphorescence emission wavelength of F or FITC, with the results agreed well with those obtained by enzyme-linked immunoassay (ELISA). Meanwhile, the mechanisms for the labeling reaction and the sensing detection of AFP-V were discussed.
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Introduction

Hepatocellular carcinoma (HCC) is one of the most familiar malignant diseases all over the world. HCC is closely related to the content of AFP-V. It was reported by Sato et al. [1] that the cirrhosis patients whose AFP-V content increased would mostly be diagnosed as suffering hepatocellular carcinoma in 3–18 months. If AFP-V is used to forecast hepatocellular carcinoma, the accuracy can reach 94% [2]. At present, the available methods for the diagnosis of HCC including immunohistochemical staining [3], fluorescence diagnosis [4], magnetic resonance imaging [5] and three-dimensional conformal radiotherapy [6]. There are many methods for the determination of AFP-V reported both at home and aboard, such as affinity-electrophoresis enzyme immunoassay (detection limit (L.D) = 1.0×10^{-9} g mL^{-1}) [7], affinity-absorbent assay (LD = 9.0×10^{-10} g mL^{-1}) [8], time resolved fluoroimmuoassay (LD = 1.0×10^{-10} g mL^{-1}) [9], antibody affinity-blotting method (LD < 4.0×10^{-8} g mL^{-1}) [10], etc. Though the LDs of above methods are at ng level, they have been rarely applied to clinic diagnosis [10]. Obviously, it is of high academic research value and application prospect to search for a new method with high sensitivity and accuracy to determine AFP-V and forecast HCC in the field of life sciences.

In recent years, lectin has been used as a glycosyl probe in the research of the glycoprotein, glycolipide, glycosyl-chain structure and alkaline phosphatase (ALP) [11]. For example, ELISA has been used to determine ALP [12]. Therefore, the exploitation of a high efficient labeling reagent of lectin is the key of biology analysis. The fullerene ($C_{50}Cl_{10}$) and its derivatives have the luminescent characteristics [13]. It has been reported that the fluorescence signal of C_{60} was enhanced sharply by modifying it with DMA [14]. There have been also many reports on the synthesis of multi-hydroxyl C_{60} derivatives, F derivatives, aminophenol derivatives and dendritic fullerene derivatives [15–17] as well as the fluorescent property of water-soluble F [18]. The ALP [19,20], glucose [21], As (V) [22] and Mn^{2+} [23] were detection based on phosphorescent property of F, showing the broad analytical application prospects of F. Inspired by these reports, we designed this phosphorescent sensor for the determination of trace AFP-V and the forecast of human diseases based on the amplification effect of DMA on room temperature phosphorescence (RTP) of F and FITC.

In this work, new DMA-F-FITC phosphorescent labeling reagent and phosphorescent sensor were developed. The sensitivity (LD: 1.2×10^{-13} g mL^{-1} for F and 9.0×10^{-14} g mL^{-1} for FITC, sandwich way) of this phosphorescent sensor was higher than those of Refs. [7–10]. New sensor was suitable for the determination of ultra-trace AFP-V in serum and the clinical diagnosis for HPC. Compared with ELISA, this sensor except a batch of phosphorescent sensor were prepared for use under room temperature, it has many merits also, such as the higher sensitivity, needs only microliter samples; many operations, including suspending samples, washing, drying and the measurement of phosphorescence can be easily conducted on many kinds of substrates, are similar to those of ELISA showing its broad application prospect. This study not only opened up the analytical application of sensor, F and lectin, but also provided a new way for the development of SS-RTP.

Experimental

Apparatus and reagents

Phosphorescent measurements were carried out on a Perkin–Elmer LS-55 luminescence spectrophotometer with a solid surface analysis apparatus (Perkin Element Corporation of US). 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}. KQ-250B ultrasonic washing machine (Kunshan Ultrasonic Machine Company) and AE240 electronic analytical balance (Mettler-Toledo Instruments Company Limited) were also used. A 0.50-μL flat head micro-injector (Shanghai Medical Laser Instrument Plant, China) was used to introduce the solution of μL level.

AFP-V, bovine serum albumin (BSA) and Con A were all purchased from Sigma Corporation and stored at 0–4 °C. They were diluted to 1.00 pg mL^{-1} and 100.00 pg mL^{-1} AFP-V (diluted with 0.10 mol L^{-1} Na_2CO_3–$NaHCO_3$ buffer solution of pH value being 9.40) 700.0 ng mL^{-1} Con A (diluted with 0.067 mol L^{-1} KH_2PO_4–Na_2HPO_4 buffer solution gradually of pH value being 7.4) and 10 mg mL^{-1} BSA (diluted with 0.10 mol L^{-1} Na_2CO_3–$NaHCO_3$ buffer solution), respectively. 1.0×10^{-5} mol L^{-1} F (C_{60} Fullerol with 24–26 hydroxy groups was synthesized directly by the reaction of fullerene with aqueous NaOH and H_2O_2 in the presence of tetrabutylammonium hydroxide as the catalyst [24]), 1.0×10^{-5} mol L^{-1} FITC, 0.10 mol L^{-1} DMA, 0.10 mol L^{-1} Na_2CO_3–$NaHCO_3$ buffer solution, 0.067 mol L^{-1} KH_2PO_4–Na_2HPO_4 buffer solution, 0.050 mol L^{-1} Tris (trihydroxymethyl aminomethane)-HCl buffer solution, Tris–HCl-0.1% Tween-20 washing buffer solution, 1.0 mol L^{-1} $Pb(Ac)_2$ solution and 2.0 mol L^{-1} HAc were also used in this experiment. Preparation of1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC, Alfa Company)-N-hydroxysucci-nimide (NHS, Alfa Company) coupling agent solution: the mixture of 5 mM EDC and 5 mM NHS was prepared with 40% ethanol. All reagents were AR grade except that BSA was a biological reagent. The water for experiment was thrice-distilled water.

Polyamide membrane (PAM), acetyl cellulose membrane (ACM) and nitrocellulose membrane (NCM) were purchased from luqiaosijia biochemical plastic plant. The paper sheets were pre-cut into wafers for preparation (diameter was 1.5 cm) and indented (diameter was 0.4 cm.) before use.

Experimental method

Preparation of phosphorescent sensor

A 0.40 μL drop of different concentrations Con A (diluted with KH_2PO_4–Na_2HPO_4 buffer solution of pH = 7.4, gradually) was suspended onto the indentation of ACM wafers by a 0.50-μL flat head micro-injector, and then stored at 4 °C overnight. The substrate wafer was immersed in BSA solution at 37 °C for 0.5 h, then washed with washing buffer solution by ultrasonic oscillation for 3 times repeatedly (20 mL of washing solution at a time, wash for 3 min), sipped up with filter paper. Took ACM out, and then 0.40 μL labeling reagents (1.50 mL of 1% (V/V) DMA-0.40 mL of 1.0×10^{-5} mol L^{-1} F-5.00 mL of 1.0×10^{-5} mol L^{-1} FITC ethanol solution) were added on the center indentation of ACM. Then, a 0.40 μL drop of EDC-NHS solution was suspended onto the center indentation of ACM, after reacting at 37 °C for 2 h, the labeling product (DMA-F-FITC-Con A) was obtained by the reaction between Con A and DMA-F-FITC. Then took out the labeling product and repeatedly washed for three times by ultrasonic oscillation to eliminate the remained marker reagent on ACM, sipped up with filter paper and phosphorescent sensor was obtained for use. The phosphorescent sensor not only could be batch prepared, but it was used under room temperature. The optimal concentration of Con A was examined by the measurement results of the affinity adsorption (AA) reaction between DMA-F-FITC-Con A phosphorescent sensor and different concentrations of AFP-V.

Affinity adsorption reaction and sensor detection for AFP-V

The type of AA reaction used in this paper was sandwich way. A 0.40 μL drop of Con A was suspended onto the indentation of ACM wafers membrane (Φ = 4 mm) by a 0.50 μL micro-injector, then

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