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An environment-sensitive fluorescent probe for quantification of human serum albumin: Design, sensing mechanism, and its application in clinical diagnosis of hypoalbuminemia

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ABSTRACT

Unusual human serum albumin concentration in serum or urine represents an important early biomarker for various medical conditions and diseases. Therefore, accurate quantification of HSA concentration in real biological samples has gained great importance for clinical diagnosis and preventive medicine. However, most of previous reports focus on detection of serum samples collected from healthy individuals, rather than the patients with abnormal serum albumin level. Further validation of HSA probes in clinical diagnosis is urgently needed yet remains challenging. Here, we reported an environment-sensitive fluorescent probe **CD1**, which exhibited fast response, high sensitivity, and excellent selectivity towards HSA. The limit of detection was determined to be as low as 8.6 nM (0.57 mg/L). Based on this method, the quantitative determination of HSA in real serum samples from both healthy individuals and patients with hypoalbuminemia are firstly reported, which demonstrated its practical application in clinical disease pre-diagnosis.

1. Introduction

Human serum albumin (HSA) is the most abundant protein in blood plasma ranging from 35 to 55 g/L in a healthy adult, which takes up approximately two thirds of the whole serum proteins [1]. In circulatory system, HSA presents several pivotal functions, including transportation of endogenous and exogenous ligands (free fatty acids, thyroxine, metabolites, etc) regulation of colloid osmotic pressure, the free radical scavenging, as well as anti-oxidant properties [2,3]. In hematologic examination, unusual HSA concentration in serum or urine represents an important early biomarker for cardiovascular disease, diabetes, kidney disease, etc. [4,5] For example, hypoalbuminemia as a medical sign, defined by a serum albumin below than 35 g/L, is frequently observed in hospitalized patients [6]. Hypoalbuminemia is commonly associated with several pathological conditions, such as innutrition, rheumatoid arthritis, sepsis, infection, and cancer [7]. Moreover, preoperative hypoalbuminemia is the efficient predictor of mortality and morbidity after surgery [8]. Therefore, accurate quantification of HSA concentration in real biological samples has gained great importance for clinical diagnosis and preventive medicine.

To date, several detection methods for HSA have been extensively studied, such as immunoassays [9,10], surface-enhanced Raman scattering (SERS) [11], LC-MS/MS proteomic [12], capillary electrophoresis [13], and colorimetric methods (*e.g.* BCG [14]). These methods, however, either exhibit low sensitivity and selectivity in responding to proteins, or suffer from the high cost and lengthy procedures. Over last few decades, the fluorescent detection assay has drawn a lot of attentions owing to its simplicity, high sensitivity, and real-time response features [15–17]. A number of fluorescent probes for HSA has been designed and synthesized, and remarkably, few of recent studies achieve the HSA detection qualitatively and quantitatively in human serum plasma [18–24] or urine [25–28]. However, since most of previous reports focus on the serum samples collected from healthy individuals, rather than the patients with abnormal serum albumin level, further validation of HSA probes in clinical diagnosis is urgently needed yet remains challenging.

In our previous works, we found that the donor-π-acceptor (D-π-A) structured fluorophores with environment-sensitive characteristics

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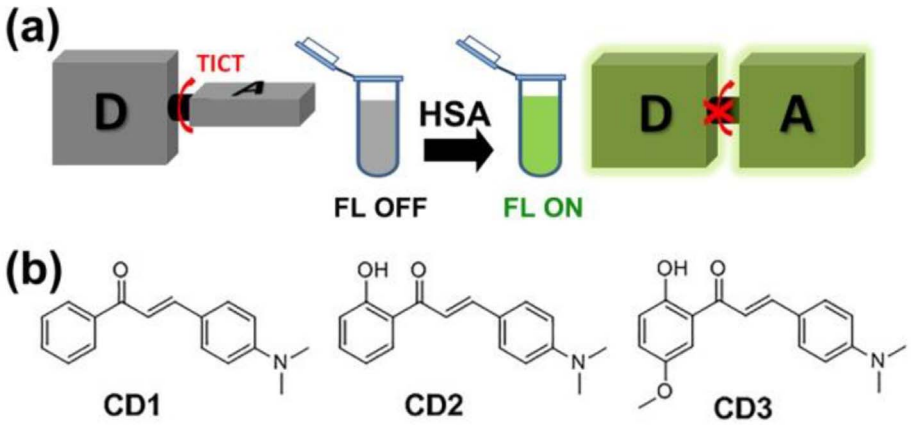

Scheme 1. (a) Design principle of D-π-A structured probe for HSA detection and (b) molecular structures of fluorophores **CD1-3**.

could be applied as the fluorescence probes for serum albumin [29,30]. As shown in Scheme 1, the fluorescence of D-π-A fluorophores is initially quenched in pure water due to the twisted intramolecular charge transfer (TICT) of *para*-substituted benzene structure. Once upon binding with serum albumin, the TICT process of fluorophore is severely restrained by the low-polar protein pocket, resulting in a significant fluorescence enhancement. Inspired by this finding, herein, three fluorescent chalcone-based dyes **CD1-3** with D-π-A structures were designed, as shown in Scheme 1, which were synthesized by one-step condensation. The **CD1-3** exhibited remarkable environmental sensitivities, and their fluorescent properties could be tuned by the solvents polarity and viscosity. Among these newly developed probes, **CD1** exhibits fast response, high sensitivity, excellent selectivity towards HSA, and high resistance in complex biological environment. Importantly, the quantitative determination of HSA in real serum samples from healthy individuals and patients with hypoalbuminemia are both achieved by using **CD1** as the probe, which demonstrates its potential application in clinical disease pre-diagnosis.

2. Experimental

2.1. Materials and instruments

All chemicals and biological analytes including human serum albumin (HSA), glutathione (GSH), homocysteine (Hcy), RNA (Ribonucleic Acid), cysteine (Cys), Ribonuclease, lysozyme, trypsin, lipase were purchased from Sigma-Aldrich and used without further purification. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVANCE III 600-MHz spectrometer. The UV-vis spectra were obtained by a Thermo-fisher Evolution 220. The fluorescent spectra were recorded by a Thermo Lumina Fluorescent spectrometer with slits width set at 5 nm for both excitation and emission. The fluorescence decay profiles were measured by Horiba DeltaFlex.

2.2. Synthesis

General synthetic methods: Equal amount (mol) of acetophenone derivatives and 4-(dimethylamino)benzaldehyde were stirred in ethanol with addition of aqueous sodium hydroxide solution. The mixture was reacted for 24 h at room temperature before being neutralized to pH 7.0 using diluted HCl (1M). The excessive solvent was removed using reduced pressure to yield the crude target product, which was further purified by silica column chromatography to obtain **CD1-3**. The detailed synthetic routes and characterization data of **CD1**, **CD2**, and **CD3** were shown in Figs. S1–S6 in Supporting Information (SI).

2.3. Polarity parameters, $E_T(30)$

$E_T(30)$ values are evaluated based on pyridinium N-phenolate betaine dye [31], and are simply defined, in analogy to Kosower's *Z* values [32,33], as the molar electronic transition energies (E_T) of dissolved dye, measured in kilocalories per mole (kcal/mol) as following equation: $E_T(30)$ (kcal/mol) = $28591/\lambda_{max}$ (nm) [34], where λ_{max} is the wavelength of the maximum of absorption band of indicator dye.

2.4. General detection methods

The fluorescent probes were firstly dissolved in THF (1 mM) as stock solution before use. For HSA titration experiments, 10 μL of stock probe solution was added into 1 mL PBS buffer (pH = 7.4), and then the high concentrated protein solution (0.1–10 mM) was successively titrated into 1 mL of testing buffer. The fluorescent spectra were collected after around 10 s mixing.

2.5. Limit of detection (LOD)

The detection limit was calculated by using 3σ/k rule based on titration experiments. Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus HSA concentration.

2.6. Molecular docking

The 3D geometry of the molecules were constructed using the Gaussian viewer, then optimized at the level of B3LYP/6-31g* with PCM implicit water solvent model. The crystal structure of HSA (PDB ID: 4K2C) was taken from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The R-value and the resolution of the file were 0.213 and 3.23 Å, respectively. Flexible ligand docking was performed by AutoDock 4.2 molecular docking program using the implemented empirical free energy function and the Lamarckian Genetic Algorithm.

The Autogrid was used to calculate Grids. The grid spacing was 0.375 Å as default. 10 docking runs with 25,000,000 energy evaluations were performed. The output from AutoDock was rendered with PyMol and the resulting docking solutions were subsequently clustered with a root mean square deviation tolerance of 2 Å and were ranked by binding energy values.

2.7. Job's plot

The fluorescence intensity of different ratios of **CD1**-HSA mixture in PBS buffer (pH ~ 7.4) were recorded while the overall concentrations of the mixture remained at 10 μM to determine the stoichiometric point.