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Fluorescent polarity probes for identifying bovine serum albumin: Amplification effect of para-substituted benzene

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ABSTRACT

Fluorescent probes **1**–**3** with coumarin as the fluorophore were designed and synthesized for the determination of bovine serum albumin (BSA). All three probes exhibited evidently solvatochromic UV–vis and fluorescence spectra. Compound **3** was the most effective towards the solvent's polarity: 155 nm (vs. 60 nm for **1** and 100 nm for **2**) red shift in the emission maximum was found as the solvent changing from cyclohexane to phosphate buffer solution. These compounds were applied to detect BSA based on the hypothesis that the polarity of the microenvironment surrounding the probe will undergo significant change when the probe moves from the bulk solution to the hydrophobic domains of BSA. **3** was the most sensitive towards BSA and the detection limit of BSA was 0.6 μg/mL with **3** as the probe, which ensured the detection of BSA content in fetal bovine serum with good recovery.

1. Introduction

Serum Albumin (ALB) is the most abundant protein in blood plasma, which plays an important role in maintaining plasma osmotic pressure, transporting a wide variety of compounds and balancing nutrition [1,2]. Low levels of ALB may indicate liver and kidney troubles or malnourishment caused by low protein diet [3,4]. It is necessary to measure ALB content in blood plasma or other biological fluids to obtain useful information about a patient's health. Bovine serum albumin (BSA) is frequently studied as a model protein because of its structural homology with human serum albumin (HSB) [5]. Therefore, the development of analytical methods for BSA detection in biological samples has attracted much attention [6–9].

There are several hydrophobic cavities inside BSA, while its surface is hydrophilic and negatively charged at neutral pH [10]. Consequently, the polarity in the bulk solution and that in the hydrophobic core of BSA are quite different. Organic dyes often bind in the hydrophobic domain of BSA, hence, the polarity of the environment surrounding the dye molecules is expected to undergo dramatical change upon the addition of BSA. Accordingly, obvious shifts in the absorption and emission bands of a polarity-sensitive dye could be induced by the variation of the microenvironment's polarity. Therefore, the photophysical properties of the dye change steadily with increasing BSA concentration. On account of the above mentioned hypothesis, many probes were explored to determine BSA based on the lower polarity inside BSA: 1-anilino-8-naphthalene sulfonate is a commonly used probe in the investigation of the conformation and fold of BSA [11–15]; squaraine dyes are another kind of frequently-used probes for BSA detection [16–20]; Arai et al. developed a quinoxaline-based fluorescent polarity probe for BSA determination [21]; some research groups detected BSA based on aggregation-induced emission [22–26]. Zhang et al. quantified BSA using the deprotection of a carbamate group assisted by BSA [27].

Many polarity probes are based on a large difference in dipole moments of the ground state and the excited state. From this point of view, we designed a new probe **3** (Scheme 1) for polarity, and further applied it in BSA detection due to the lower polarity inside BSA cavity. Compounds **1** and **2** (Scheme 1) were synthesized as references. There are several common features for these three compounds: (1) using polarity sensitive coumarin as the fluorophore; (2) undergoing intramolecular charge transfer (ICT) from N,N-diethyl amino to the carbonyl group, and their photophysical properties are supposed to be influenced by the solvent's polarity. The main differences among these compounds are: (1) compounds

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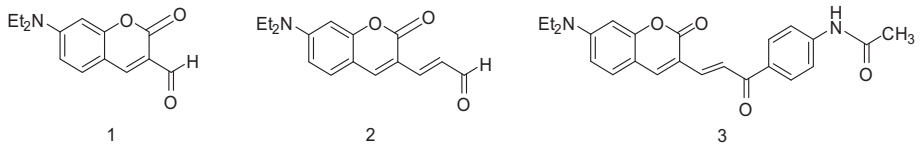


Scheme 1. The chemical structures of compounds **1**–**3**.

2 and **3** have extended conjugation systems, which lead to absorption and emission maxima at longer wavelengths; (2) a para-substituted benzene is incorporated into compound **3**, which may induce a twisted intramolecular charge transfer (TICT) state, and the spectral properties of **3** is expected to be quite different from those of **2**. We will demonstrate that **3** is highly solvatochromic and a very good probe for BSA, and therefore a potential probe for ALB.

2. Experimental

2.1. Materials and reagents

All chemicals were purchased from Aladdin Corporation and were used without further purification. Ultra-pure water was prepared through Sartorius Arium 611DI system. Fetal bovine serum was purchased from the JONLN industrial Co., LTD, Shanghai.

2.2. Synthesis

2.2.1. Compounds *1* and *2*

Compounds **1** and **2** were synthesized (Scheme 2) according to the references [28,29]. **1** (yield: 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.12 (s, 1H), 8.26 (s, 1H), 7.42 (d, J = 9.0 Hz, 1H), 6.64 (dd, J = 2.5, 9.0 Hz, 1H), 6.49 (d, J = 2.3 Hz, 1H), 3.48 (q, J = 7.1 Hz, 4H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 187.8, 161.7, 158.8, 153.4, 145.2, 132.4, 114.2, 110.1, 108.1, 97.1, 45.2, 12.3.

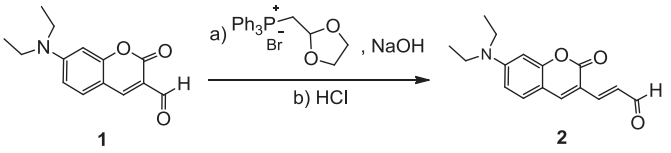
2 (yield: 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.56 (d, J = 7.8 Hz, 1H), 7.75 (s, 1H), 7.36 (d, J = 15.8 Hz, 1H), 7.26 (d, J = 8.9 Hz, 1H), 6.93 (dd, J = 7.8, 15.8 Hz, 1H), 6.55 (dd, J = 2.0, 8.9 Hz, 1H), 6.41 (d, J = 2.0 Hz, 1H), 3.39 (q, J = 7.2 Hz, 4H), 1.19 ~ 1.15 (t, J = 7.1 Hz, 6H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 193.9, 160.1, 157.0, 152.2, 147.1, 143.6, 130.3, 128.5, 113.9, 109.6, 108.5, 97.0, 45.0, 12.4.

2.2.2. Probe *3*

Compound **3a** (Scheme 3): Acetylchloride (540 mg, 6.88 mmol) was added dropwise to anhydrous dichloromethane solution of 4-aminoacetophenone (500 mg, 3.7 mmol), and the mixtures were stirred overnight in ice bath. The solvent was evaporated under vacuum, then the crude product was purified through column chromatography (DCM:MeOH = 50:1/v:v) to give compound **3a** as white powder.

3a (yield: 41%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (s, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.7 Hz, 2H), 2.52 (s, 3H), 2.09 (s, 3H).

Probe **3** (Scheme 3): Compound **1** (150 mg, 0.61 mmol) and **3a** (163 mg, 1.21 mmol) were added to 20 mL of the mixed solvent [CH_2Cl_2/CH_3CH_2OH = 1:1 (v/v)], then 10 drops of pyrrolidine were dropped into the above solution. The mixture was stirred at 35 °C for 2 d. After evaporation of the solvent under reduced pressure, the crude product was purified using column chromatography (PE:DCM = 1:1 ~ DCM:MeOH = 200:1) to give red solid **3**.



Scheme 2. The synthesis of compound **2**.

3 (yield: 24%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.25 (s, 1H), 8.33 (s, 1H), 8.04 (d, J = 15.4 Hz, 1H), 8.00 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 15.4 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 6.73 (dd, J = 2.1, 9.0 Hz, 1H), 6.55 (d, J = 2.1 Hz, 1H), 3.49 (q, J = 7.0 Hz, 4H), 2.12 (s, 3H), 1.20 (t, J = 7.0 Hz, 6H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 187.8, 169.1, 160.2, 156.7, 152.1, 145.6, 143.8, 139.0, 132.8, 130.7, 129.6, 121.5, 118.7, 114.1, 109.9, 108.8, 96.6, 44.8, 24.4, 12.6; HRMS (ESI) m/z calcd for $C_{24}H_{25}N_2O_4$ $(M+H)^+$: 405.1814, Found 405.1813.

2.3. Spectral measurements

Accurately weighted amount of the dyes **1**–**3** were dissolved in DMF to obtain 3×10^{-2} M stock solution. The stock solution was diluted with corresponding solvents to acquired 10 μM dye solutions. Absorption spectra were measured with an Evolution 220 UV–Visible spectrophotometer (Thermo Scientific). Fluorescence spectra were carried out in a Lumina Fluorescence Spectrometer (Thermo Scientific). Fluorescence quantum yield was determined with coumarin 153 (Φ_f = 0.38 in ethanol) as the reference [30]. NMR spectra were performed with a Bruker AV-400 spectrometer (400M Hz). Mass spectra were recorded on a MA 1212 Instrument using standard condition (ESI, 70 eV).

Time-resolved fluorescence data were acquired with an Edinburgh FL 900 Fluorescence Spectrometer equipped with a laser (λ_{ex} = 441 nm). A di-exponential function was used to fit the fluorescence decay.

2.4. BSA titration

A series of BSA phosphate buffer solutions (20 mM, pH 7.4) with different concentrations (2 μg/mL ~ 10 mg/mL) were prepared. 10 μL of the dye stock solution were added to 3 mL of the BSA solution to keep [dye] = 10 μM.

2.5. Measurement of the spiked BSA in fetal bovine serum

Fetal bovine serum (FBS) was diluted 30 times with phosphate buffer solution. 1.5 mL of diluted FBS was mixed with 1.5 mL of buffer solution/or 0.75 mL buffer solution and 0.75 mL of 1.0 mg/mL BSA stock solution/or 1.5 mL of 1.0 mg/mL BSA stock solution. Then 10 μL of the dye stock solution was added to the above prepared FBS solutions.

2.6. Determination of the detection limit

The detection limit (LOD) was obtained by $3S_b/k$, where S_b is the standard deviation of the blank measurements of 10 times, and k is the slope of the fitted line.