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A novel fluorescent probe based on β-*C*-glycoside for quantification of bovine serum albumin

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ABSTRACT

Two novel *C*-glycoside-coumarin fluorescent dyes are reported and their UV/Vis, fluorescence spectra and fluorescence-response to bovine serum albumin (BSA) are investigated and discussed. The fluorescence emission is red-shifted and quenched with the increase in polarity of solvents. Moreover, the fluorescence intensity of the dyes increased upon the addition of BSA in pH 7.4 phosphate buffer solution. An excellent linear relationship (R^2 = 0.99495) for compound **1** was obtained between fluorescence intensity and bovine serum albumin concentration. The method show a good selectivity and sensitivity for BSA over few proteins, other ions and amino acids, which was suggested as high selective and sensitive fluorescent reagents for quantification of BSA in biological science.

1. Introduction

The interactions between biological macromolecules and small molecules have attracted great interest in recent years [1–3]. Among bio-macromolecules, Serum albumin is the most abundant protein in the animal's circulatory system [4,5]. Low levels of ALB may indicate liver and kidney troubles or malnourishment caused by low protein diet [6,7]. 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), as one of the major components in plasma protein (0.38 g mL^{-1}), is most extensively studied, due to its availability, low cost, stability, unusual binding properties and structural homology with human serum albumin (HSA) [8,9]. Therefore, detecting BSA in whole blood, blood plasma and other biological fluids is routinely involved in biochemistry, clinical medicine and library tests. Searching for a sensitive method for the determination of BSA is an ongoing subject of investigation. Among various detection methods, fluorescent sensors have exhibited advantages in terms of sensitivity and selectivity. Therefore, developing fluorescent sensors for various proteins has gained increasing attention.

The spectroscopic methods that are based on an intermolecular probe (a probe that binds to the protein and is not the natural cofactor of it) can be divided to two main methods: (1) Förster (or fluorescence) resonance energy transfer (FRET) which describes a process where the light excitation of a donor chromophore induces the radiative fluorescence of an acceptor chromophore. (2) Molecular probes that change their fluorescence intensity upon binding to proteins. This class of intermolecular probes includes a wide variety of molecules, where the common ones are naphthalene sulfonate derivatives, thioflavins, nilered, rhodamines, squaraines and cyanines [10,11].

The main focus of our current research has been concentrated on carbohydrate, including the synthesis of natural products, fluorescence sensor, drug intermediate [12]. Carbohydrate derived fluorescent probes, as neutral, water-soluble, and good biocompatible have gained much attention for probe for ions [13–16], interact with proteins [17–20] and biological imaging [21,22]. In addition, *C*-glycosides catalyzed by base [23] and Lewis acid [24] in one step have been investigated by our group. On the basis of that, in the present study, we described the synthesis and spectroscopic characterisation of water-soluble dyes **1** and **2** incorporate two different *C*-glycosides and coumarin (Fig. 1). We also studied their spectral and photophysical properties in organic solvents or in phosphate buffer solution (PBS) in the absence and presence of BSA. The results indicated that the dyes, which prepared with

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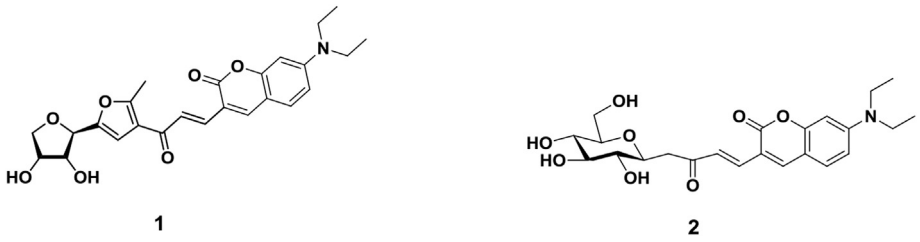

Fig. 1. The chemical structures of compound **1** and **2**.

satisfactory yields, had the potential for quantification of bovine serum albumin.

2. Experimental

2.1. General information and materials

All reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. Melting points (m.p.) were determined on a WRS-1B digital melting-point apparatus (Shanghai Shenguang Instrument Co., Ltd). ^{1}H and ^{13}C NMR spectra were measured on Bruker Avance III 500 MHz spectrometer. Fluorescence spectra were obtained by using a Shimadzu RF-5301PC Fluorescence Spectrometer equipped with a xenon lamp, 1.0 cm quartz cells. Absorption spectra were measured with Lambda 35 UV-vis spectrophotometer. Emission and absorption spectra were measured on fluorescence and absorption spectrometers, respectively. All the solvents used were of analytical grade. Ultra-pure water was prepared through Sartorius Arium 611DI system. Reactions were monitored by thin-layer chromatography using silica gel coated TLC plates and detection was performed by UV absorption (254 nm) where applicable, and by spraying with 50% sulfuric acid in ethanol followed by charring. All measurements were carried out at room temperature (~298 K).

2.2. Synthesis

2.2.1. Synthesis of C-furyl glycoside (*3*)

To a solution of glucose **6** (1.8 g, 10 mmol) and acetylacetone (1.54 mL, 15 mmol) in water (50 mL) was added $CoCl_2$ (10%, 0.13 g) at room temperature. The reaction mixture was stirred and refluxed at 90 °C for 8 h. After completion of the reaction as indicated by TLC, the reaction mixture was extracted by ethyl acetate, and the organic layer was evaporated to dryness under reduced pressure. The residue was purified by recrystallization using petroleum ether and ethyl acetate and furnished pure compound 2 (1.7 g, 87%). yellow powder; m.p.: 102–104 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (s, 1H), 4.61 (d, J = 6.2 Hz, 1H), 4.32 (t, J = 4.6 Hz, 2H), 4.19 (dd, J = 10.0, 4.3 Hz, 1H), 3.85 (dd, J = 13.0, 3.7 Hz, 2H), 3.76 (s, 1H), 2.52 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 193.9, 158.2, 149.2, 121.1, 108.4, 75.9, 73.7, 72.1, 70.0, 28.1, 13.6.Anal. Calcd for $C_{11}H_{14}O_5$: C, 58.15; H, 6.43. Found: C, 58.22; H, 6.35.

2.2.2. Synthesis of C-pyranyl glycoside (*4*)

Glucose **6** (10 mmol), acetylacetone (1.54 mL, 15 mmol), sodium bicarbonate (168 mg, 2 mmol), and 50 mL H_2O were introduced and mixed in a 50 mL single-necked flask equipped with a reflux condenser. The reaction mixture was stirred and refluxed at 90 °C for 12 h. The solutions were allowed to cool to r.t and treated with cation exchange resin (sodium form) to reach pH 5. The resin was filtered. The aq soln was washed with CH_2Cl_2 and concentrated. The products were used without further purification. A solution of the product of last step (10 mmol), NaOAc (1.2 g, 15 mmol) was added Ac_2O (10 mL), and stiring at 135 °C for 4–6 h. This mixture was extracted with ether which was then washed with sodium bicarbonate solution, dried, and stripped of solvent in vacuum. Purification of the residues was performed by recrystallization from EtOH to afford compound **2** as a white power (yield 88%), m.p.: 97–99 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.16 (t, J = 9.4 Hz, 1H), 5.00 (t, J = 9.7 Hz, 1H), 4.85 (t, J = 9.7 Hz, 1H), 4.20 (dd, J = 12.4, 4.9 Hz, 1H), 4.00 (dd, J = 12.4, 2.1 Hz, 1H), 3.95 (td, J = 9.4, 3.0 Hz, 1H), 3.65 (ddd, J = 10.0, 4.9, 2.2 Hz, 1H), 2.70 (dd, J = 16.6, 8.9 Hz, 1H), 2.44 (dd, J = 16.6, 3.0 Hz, 1H), 2.14 (s, 3H), 2.03 (s, 3H), 1.99 (dd, J = 9.3, 0.9 Hz, 6H), 1.95 (d, J = 6.8 Hz, 3H).^{13}C NMR (125 MHz, $CDCl_3$) δ 205.0, 170.6, 170.2, 169.8, 169.5, 75.6, 74.0, 73.7, 71.4, 68.3, 61.9, 45.2, 30.9, 20.5.Anal. Calcd for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.23. Found: C, 52.48; H, 6.30.

2.2.3. Synthesis of compound *5*

To a solution of compound **4** (0.19 g, 0.5 mmol) in CH_2Cl_2 (5 mL) were added pyrrolidine (30 mol %) and 7-N, N-Diethylaminocoumarin-3-aldehyde (0.15 g, 0.6 mmol). After stirring at room temperature for 24 h, the reaction mixture was evaporated under reduced pressure and extracted by EtOAc-water. The EtOAc layer was dried by Na_2SO_4 and concentrated. The product was purified by column chromatography to give compound 5 as a yellow solid, yield 0.25 g (84%). m.p.: 101–103 °C. ^{1}H NMR (500 MHz, CDCl3) δ 7.83 (s, 1H), 7.52 (d, J = 15.8 Hz, 1H), 7.39 (d, J = 8.9 Hz, 1H), 7.34 (d, J = 2.3 Hz, 1H), 6.68 (dd, J = 8.9, 2.0 Hz, 1H), 6.56 (s, 1H), 5.29 (t, J = 9.3 Hz, 1H), 5.16 (t, J = 9.7 Hz, 1H), 5.05 (t, J = 9.7 Hz, 1H), 4.33 (dd, J = 12.3, 4.5 Hz, 1H), 4.26–4.15 (m, 1H), 4.11 (d, J = 12.3 Hz, 1H), 3.79 (dd, J = 9.9, 2.3 Hz, 1H), 3.52 (q, J = 7.0 Hz, 4H), 3.05 (dd, J = 16.4, 8.5 Hz, 1H), 2.74 (dd, J = 16.4, 3.0 Hz, 1H), 2.28–1.98 (m, 12H), 1.31 (t, J = 7.1 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.5, 169.8, 169.3, 168.8, 168.6, 159.3, 155.8, 151.1, 144.2, 137.4, 129.1, 125.4, 113.3, 108.6, 107.8, 96.0, 74.7, 73.4, 73.2, 70.7, 67.6, 61.1, 44.1, 42.4, 19.7, 11.5.Anal. Calcd for $C_{32}H_{38}O_{12}$: C, 62.53; H, 6.23. Found: C, 62.58; H, 6.15.

2.2.4. Synthesis of probe *1*

To a solution of compound **3** (0.08 g, 0.3 mmol) in CH_2Cl_2 (5 mL) were added pyrrolidine (30 mol %) and 7-N, N-Diethylaminocoumarin-3-aldehyde (0.10 g, 0.4 mmol). After stirring at room temperature for 24 h, the reaction mixture was evaporated under reduced pressure and extracted by EtOAc-water. The EtOAc layer was dried by Na_2SO_4 and concentrated. The product was purified by column chromatography to give compound **1** as a yellow solid. m.p.: 122–124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 15.1 Hz, 1H), 7.76 (s, 1H), 7.52 (d, J = 15.2 Hz, 1H), 7.33 (d, J = 8.7 Hz, 1H), 6.83 (s, 1H), 6.61 (s, 1H), 6.50 (s, 1H), 5.35 (s, 1H), 4.78–4.61 (m, 1H), 4.43 (s, 2H), 4.27 (s, 2H), 3.91 (s, 1H), 3.45 (d, J = 6.8 Hz, 4H), 2.56 (dd, J = 56.6, 24.4 Hz, 4H), 2.35 (d, J = 32.5 Hz, 2H), 2.23 (d, J = 8.4 Hz, 1H), 2.01 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.3, 158.9, 155.6, 150.9, 148.8, 145.2, 137.4, 129.0, 124.1, 121.9, 108.6, 108.4, 108.0, 96, 73.8, 72.3,70.1, 44.1, 28.7, 28.4, 28.1, 13.7,