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# Biophysical influence of coumarin 35 on bovine serum albumin: Spectroscopic study

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ABSTRACT

The binding mechanism and protein–fluorescence probe interactions between bovine serum albumin (BSA) and coumarin 35 (C35) was investigated by using UV–Vis absorption and fluorescence spectroscopies since they remain major research topics in biophysics. The spectroscopic data indicated that a fluorescence quenching process for BSA–C35 system was occurred. The fluorescence quenching processes were analyzed using Stern-Volmer method. In this regard, Stern-Volmer quenching constants ($K_{SV}$) and binding constants were calculated at different temperatures. The distance r between BSA (donor) and C35 (acceptor) was determined by exploiting fluorescence resonance energy transfer (FRET) method. Synchronous fluorescence spectra were also studied to observe information about conformational changes. Moreover, thermodynamics parameters were calculated for better understanding of interactions and conformational changes of the system.



## 1. Introduction

Coumarin 35 (7–dietil amino 4-trifloro methyl coumarin or coumarin 481) is a dye compound derived from 7–amino coumarins. The 7-amino coumarins are significant for dye laser applications, which have blue–green emission region [1]. Recently, various coumarin derivatives, which are used analgesics, anticoagulants, fluorescence probes and sensitizer in photo processes have been reported in the literature [2,3]. In this concept, coumarin 35 has been commonly studied in terms of its photophysical feature and hydrogen bonding property in various solvents [4–6].

BSA is an abundant protein in plasma and play major role to deliver fatty acids, drugs and other metabolites through the body [7,8]. Therefore, many researchers have studied to understand the structure and properties of serum albumin [9,10]. Its interaction and binding properties with other molecules and ligands have been deeply explored [11,12]. The BSA consists of 582 amino acids and shows 76% similarity to human serum albumin (HAS). The BSA has three linear site named domain I–II–III, at which each of them has two subdomains A and B. The BSA contains two tryptophan region as tryptophan 134 (trp–134) and tryptophan 212 (trp–212). The trp–134 is in the eighth helix in domain I (subdomain IB), while the trp–212 is in the second helix in domain II (subdomain IIA) [13]. The interactions and binding properties of the BSA with antitumor drugs and fluorescence probes have been reported [14,15]. Therefore, the structural characterization and molecular interactions of the BSA with the other probes has a crucial role for many research fields such as clinical medicine and life sciences. Spectroscopic techniques, for instance, UV–Vis absorption and steady-state and time-resolved fluorescence are useful to understand molecular interactions in a liquid medium due to their high sensitivity, rapidity and ease of applications. The interactions, binding effects and conformational changes of the BSA with fluorescence probes were analyzed by using the spectroscopic techniques, since photophysical properties of fluorophores are affected by changes in their surroundings.

Binding of small molecules to proteins remains a major research topic in biophysics. We have studied the binding of a photoactive dye coumarin 35 to BSA as a model to investigate biophysical effects of the 7–amino coumarins on proteins. Interaction of important biomolecules such as nucleic acids and proteins with coumarin derivatives have been successfully demonstrated by using spectroscopic techniques. The interaction of several coumarin derivatives with BSA, which are 7–amino–coumarins [16] 4–hydroxycoumarins [17], quaternizable coumarin [18], coumarin–6–yl, [19] coumarin–3–carboxylic acid [20] and substituted hydroxychromone derivatives of coumarin [21], have been investigated by using absorption and fluorescence spectroscopies. In contrast, there is no report BSA–C35 system in literature.

In the present study, the binding mechanism and molecular interactions of the BSA with coumarin 35 in an aqueous solution were examined by using UV–Vis absorption, steady–state and time–resolved fluorescence studies. The binding modes, constants, sites, fluorescence intensity quenching and intermolecular distance for the BSA–coumarin 35 system were determined.

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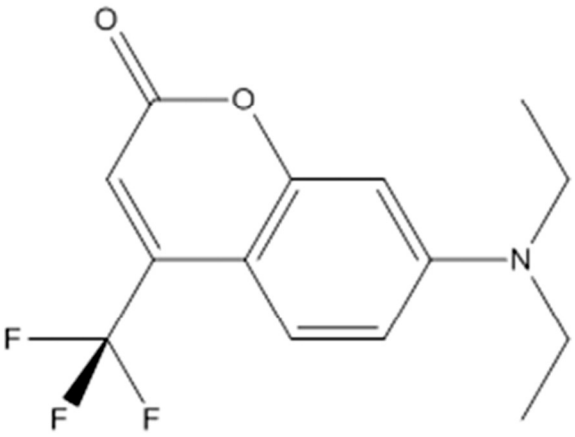


**Fig. 1.** The molecular structure of coumarin 35 compound.

## 2. Experimental

### 2.1. Materials

The spectroscopic grade 7–dietil amino 4–trifloro methyl coumarin or coumarin 35 given in Fig. 1, the BSA (66 kDa), Trizma (Tris) and NaCl (≥99.5%) were purchased from Sigma. Methanol (≥99.5%) was obtained from Merck.

### 2.2. Preparation of BSA, C35 and BSA–C35 solutions

BSA solution ($1.0 \times 10^{-5}$ M) was prepared in Tris–HCl buffer solution of pH 7.4 containing 0.15 molL$^{-1}$ Tris and 0.05 molL$^{-1}$ NaCl. C35 stock solution ($1.0 \times 10^{-3}$ M) was prepared in methanol. The desired concentrations of C35 were prepared from the stock solution via the dilution process. For the BSA–C35 solutions, the certain volume of C35 stock solution was pipette and transferred into a vial. Then, the solvent was evaporated by argon gas purging and the BSA solution was added into the vial.

### 2.3. Apparatus

UV–Vis absorption and fluorescence spectra of the samples were taken with Perkin Elmer Lambda 35 UV/VIS Spectrophotometer and Shimadzu RF–5301PC spectrofluorophotometer, respectively. The excitation and emission slits were set at 3.0 nm. The absorption spectra were recorded in the region of 220 nm to 550 nm at room temperature. For the fluorescence measurements, the C35–BSA system was excited at

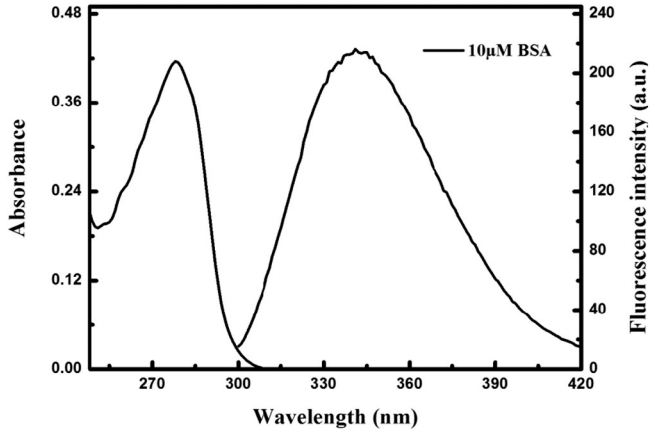


**Fig. 2.** UV–Vis absorption and fluorescence spectra of BSA of 10 μM in Tris–HCl buffer solution (pH 7.4).

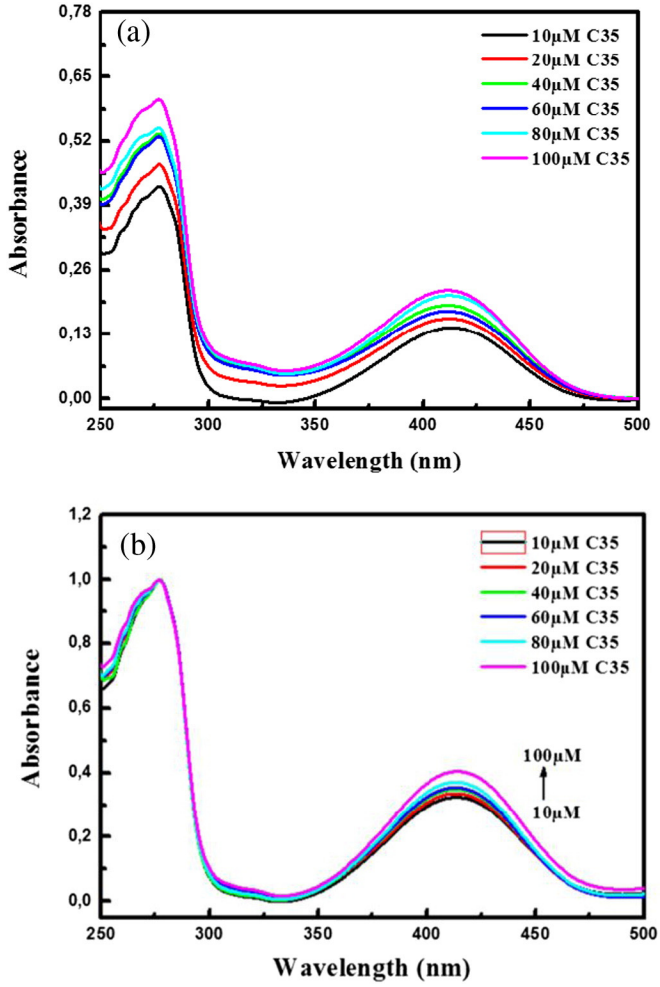


**Fig. 3.** Observed (a) and normalized at 278 nm (b) UV–Vis absorption spectra of the BSA with various concentrations of C35 at pH 7.4.

295 nm and the fluorescence spectrum was recorded between 300 nm and 585 nm at different temperatures (280, 288, 299 and 310 K). The synchronize fluorescence spectra of BSA were also recorded with the absence and presence of C35 at room temperature. For the synchronize fluorescence spectra, two different measurements were carried out. First, Δλ was 15 nm, the BSA–C35 system was excited at 265 nm and the fluorescence spectrum was recorded between 280 nm and 340 nm. The second, Δλ was 60 nm, the system was excited at 250 nm and the fluorescence spectrum was recorded between 310 nm and 370 nm.

## 3. Results and discussion

### 3.1. Spectral properties of BSA in a physiological pH

It is known that the BSA is a heart–shaped "*N* form" protein in a solution at neutral pH [22]. In protein dynamics and conformation studies, it is required to determine the fluorescence properties of the fluorophores such as tryptophan and tyrosine residues in proteins. In the literature, it is reported that fluorescence emission of BSA mainly depends on three intrinsic fluorophore molecules the tryptophan (Trp–134 and 212), tyrosine (Tyr) and phenylalanine (Phe) present in the protein [23]. For the fluorescence measurements, the excitation wavelength of 295 nm was preferred to observe the contribution from these fluorophore molecules in the BSA [24]. For this aim, UV–Vis absorption and fluorescence spectra of the BSA of $1.0 \times 10^{-5}$ M in Tris–HCl buffer solution (pH 7.4) were shown in Fig. 2.