Dyes and Pigments 142 (2017) 62–68

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Spectral-luminescent study of coumarin 35 as fluorescent "light-up" probe for BSA and DNA monitoring

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ARTICLE INFO

Article history:
Received 16 January 2017
Received in revised form
2 March 2017
Accepted 9 March 2017
Available online 12 March 2017

Keywords:
Coumarin 35
Fluorescence intensity
Scatchard method
Binding constant
Metal ion sensor

ABSTRACT

The monitoring of bovine serum albumin (BSA) and deoxyribonucleic acid (DNA) in physiological pH were studied comparatively by using coumarin 35 dye (C35). For this aim, the binding constant and binding number sites for the interactions of the dye with BSA and DNA were determined by Scatchard method. According to the spectroscopic data, fluorescence intensity of C35 was 30-fold and 8.5-fold increased with the presence of BSA and DNA, respectively. Effects of metal ions on the interactions between BSA-C35 and DNA-C35 systems were also studied in terms of metal ion sensor properties. Therefore, the effect of thirteen cations (K^+, Li^+, NH_4^+,Ca^{2+}, Ba^{2+}, Mg^{2+}, Sr^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Al^{3+}, Fe^{3+}, Sn^{4+}) on the BSA-C35 and DNA-C35 systems were investigated, the variations of binding constants were determined and comparisons were made. With this respect, this study is important in terms of gaining a new light-up probe to the literature.

1. Introduction

Bovine serum albumin (BSA) is a large globular protein (66400 Da) that fits the basic amino acid profile. BSA includes two types of tryptophan: tryptophan 134 (trp-134) and tryptophan 212 (trp-212). Trp-134 is in the 8th helix in domain I and trp-212 is in the 2nd helix in domain II [1]. Trp-212 is located in the hydrophobic cavities of the protein while trp-134 is located on the surface of the molecule [2,3]. Fluorescence of BSA mainly takes place in the hydrophobic groves in tryptophan regions in domains I and II. Similar to HSA and RSA, BSA contains high amounts of amino acids of aspartic acid (Asp), glutamic acid (Glu), alanine (Ala), leucine (Leu) and lysine (Lys) [4]. BSA and HSA have 76% of similarity. Circular dichroism (CD) measurements provided for the α-helix, β-sheet, loop and random coil content of BSA as 48.7%, 0%, 10.9%, and 30.7%, respectively [5]. Bovine serum albumin has many physical functions such as binding, communicating, and incorporating fatty acids, bilirubin, steroids, etc. into circulation. It is known that many drugs bind to serum albumin in specific. Activity of a drug depends on their ability to bind to BSA. There are many studies on major research topics (such as clinical medicine, life sciences and chemistry) which aim to illuminate the structural properties of drugs through binding them to BSA and determine their effectiveness in healing [6,7].

DNA has great importance in numerous functions that are vital to an organism, such as gene expression, gene transcription, mutagenesis, and carcinogenesis. In biological studies, interaction of DNA with metal ions has a significant role in stabilizing or destabilizing bases or base pairs. Fluorescence property of pure DNA is barely existent. Therefore, in practice, this fluorescence property cannot be made use of [8]. Especially in quantitative analysis, as the fluorescence spectrum intensity is very weak, studies are always accompanied by a fluorescent probe [9,10]. In recent years, active fluorescent probes and DNA-drug designs have been widely studied [11]. Following the interactions of these probes with DNA, an increase in fluorescence intensity was observed. Berberine [12], acridine orange [13] safranine T [14], nile blue [15], methylene blue [16], neutral red dye [17] and thionine dye [18] are some of the aromatic dye compounds of which the interaction with DNA was studied. Studies with DNA-colorant cleared the way for the determination and improvement of new functional compounds [19].

Coumarins have strong fluorescence properties in the visible region. Therefore, these dye compounds are suitable for use as colorants in dye lasers and nonlinear optical chromophores [20]. They have distinct biological activity and are agents that have potential for anticancer and anticoagulant activity. One of these dyes is coumarin 35 (C35) that is known as 7-diethylamino

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http://dx.doi.org/10.1016/j.dyepig.2017.03.019
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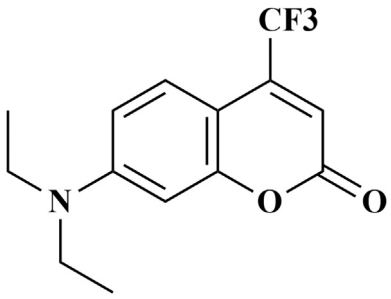

Fig. 1. Molecular structure of coumarin 35.

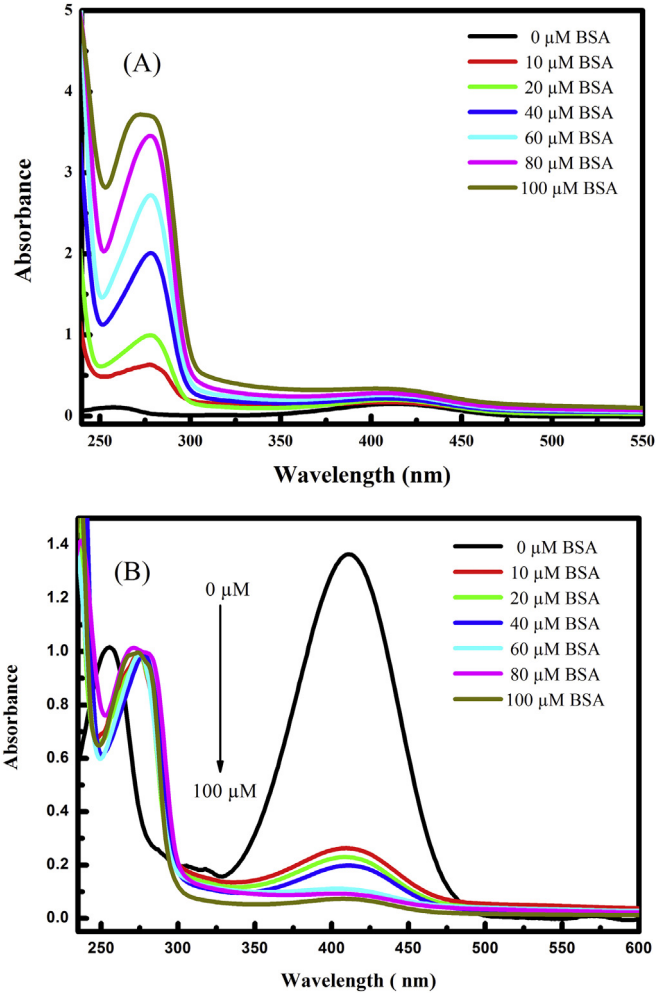

Fig. 2. a) Absorption spectra and b) Normalized absorption spectra of C35 dye of 10 μM in the media containing different concentrations of BSA.

4-trifluoromethyl coumarin or coumarin 481 (Fig. 1). The interactions between C35 and BSA have been investigated by our group with respect to the binding mechanism and molecular interactions of C35 with BSA in aqueous solution examined by UV–Vis absorption, steady-state fluorescence and time-resolved fluorescence spectroscopy techniques but not with Scatchard method [21]. On the other hand, there is no any report in the literature for the investigation of the interactions between C35 and DNA.

In the present study, the molecular interactions of C35 with BSA and DNA have been investigated by exploring Scatchard method with the aid of absorption and steady-state fluorescence spectroscopy data and, then, the influence of metal ions on these interactions was studied. The aim of working with DNA and BSA was the monitoring the response of probe for the different biological macro molecules. As a result, the fluorescence light-up probe capacity of the C35 was discussed.

2. Materials and method

2.1. Chemical materials

7-diethylamino-4-trifluoromethyl coumarin (coumarin 35; C35), bovine serum albumin (BSA; 66 kDa) to be used in experimental studies and Trizma (Tris) and sodium chloride (NaCl) compounds to be used in preparing the buffer solution were purchased from Sigma Company. As DNA, genomic mouse DNA was used. Ethanol (≥99.8%) was from Merck Company and chloride compounds of metal ions (KCl, $BaCl_2$, etc.) were purchased from Fluka Company.

2.2. Preparation of samples

The stock solutions of 1.0×10^{-3} M C35 were prepared in ethanol. BSA and DNA solutions with certain concentrations were in a Tris-HCl buffer solution with pH 7.4 which was prepared by using 0.05 $molL^{-1}$ Tris, 0.15 $molL^{-1}$ NaCl. In order to prepare C35 solutions with concentrations varying between 0.01 and 0.10 μM, first, required amount of C35 was taken from the stock solution, then, its solvent was vaporized and, then, it was added in the solutions of BSA or DNA compounds prepared in physiologic pH 7.4. The measurements were carried out for using BSA-C35 and DNA-C35 samples prepared in this manner.

2.3. Scatchard method

Scatchard method is one of the methods used in quantitative characterization in such systems as protein-ligand or hormone-ligand [22]. It is widely used method due to its easy applicability and is a useful method than others in establishing multi-site binding events. In Scatchard method, binding constants are calculated by using Equation (1).

$$r/c = nK_b - rK_b \quad (1)$$

where r is the bound ligand concentration, r/c is the bound ligand concentration per one mole of protein, K_b is the binding constant, n is the number of binding sites [23]. When determining the degree of binding of fluorophore to protein, either the maximum absorbance or fluorescence intensity values are used. In the studies,

Table 1
Spectral data and fluorescence quantum yields of BSA-C35 system (pH 7.4).

Absorbance band max. λ_{ab} (nm)		Fluorescence band max. λ_{fl} (nm)		Fluorescence quantum yields Φ_f	
Free coumarin	Bound coumarin	Free coumarin	Bound coumarin	Free coumarin	Bound coumarin
412	407	523	479	0.017 ± 0.004	0.099 ± 0.002