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Invited paper

BODIPY dyes in bio environment: Spectral characteristics and possibilities for practical application

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ABSTRACT

Boron dipyrrin (BODIPY) dyes serve as perspective fluorescent sensors and probes in a vast range of biochemical and medical applications. Thus, mechanisms of interaction with biomolecules and accompanying spectral behavior of BODIPY are of major interest for fundamental science and practical applications. 8-aryl BODIPY derivatives were found to have high fluorescent respond upon the protein addition. Investigation of the interaction between the series of BODIPY complexes and Bovine Serum Albumin (BSA) using ultraviolet-visible (UV-vis) absorption, fluorescence spectroscopy under simulated physiological conditions (pH 7.4) and molecular docking methods was performed. The results of fluorescence spectroscopy indicated that the fluorescence of hydrophobic BODIPY dyes substantially increases in the presence of BSA. The prospected reason is binding of the dye to the BSA hydrophobic cavities in the subdomain IIA. Aromatic moiety size increase causes shifting of the interaction driving force from mostly non-specific to the π-stacking. Among all probes, the 8-phenyl substituted dye showed the highest sensitivity and the quantitative fluorescent response for BSA in aqueous solution. BODIPY with 8-phenyl substituent or analogs therefore could be used as protein surface polarity sensors, at the same time all of the investigated dyes with bulky aromatic groups could be used as hydrophobic molecule tags and markers for bioimaging tasks.

1. Introduction

Fluorescence spectroscopy recently has become an upsurge of interest for practical biochemical analysis due to its non-invasive, real-time signal response, high sensitivity and selectivity [1–4]. Fluorescent proteins have revolutionized many areas of biology — e.g., fluorescent proteins can report on gene expression or protein localization in real time [5,6]. Still, lack of cell permeability of those lead to intense search for small fluorescent molecules 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene based compounds, also known as BODIPY or Boron dipyrrins are one of the most promising small fluorescent molecules for usage as optically active sensors and markers for biochemical applications [7–9]. Easiness of structural modification combined with a vast range of possible mechanisms for manifestation of sensor activity makes them perspective tool for *in vivo* and *in vitro* diagnostics [10]. Several BODIPY structures in individual state or covalently linked to organic biomolecules are commercially available and could be used without deep understanding of the nature of fluorescent respond [11,12]. Proteins, having the complex chemical and spatial structures, with a large number of centers for the universal and specific interactions are the most representative examples of biological systems, hence being the promising study objects. Several structural parameters of the fluorescent molecules should be taken into account for the protein sensing molecules design, leading to variety of possible approaches for fluorescent sensors design:

1. Combination of fluorescent core with the protein sensitive groups and substituents. Addition of electron-donor atoms and groups (amino-, carboxy-) to BODIPY core could be used to increase the number of binding sites between dye molecule and protein, thus increasing the binding constants via electrostatic interactions with protein [13–16]. Addition of aryl-substituents or alkyl chains could increase the molecule's affinity to protein through hydrophobic interactions [17,18].
2. Design of donor-π-acceptor (D-π-A) structure fluorophores providing intramolecular charge transfer (ICT) characteristics

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[19]. The fluorescent dyes undergoing ICT normally show representative solvatochromic effect, that is, the dyes may emit weak fluorescence in aqueous media, while exhibiting a dramatic fluorescence enhancement once binding with hydrophobic pocket in Human Serum Albumin (HSA). Up to now, several fluorescent probes for HSA in aqueous media have been reported, for example, aggregation induced emission (AIE) dyes [10,20].

There are several examples of BODIPY usage for protein detection using fluorescence spectroscopy [21,22] and fluorescence with absorbance both [18]. BODIPY dyes are highly fluorescent in non-polar media (yet exhibit fluorescent callback in the presence of polar molecules), have sharp and narrow emission peaks and possess reduced solvatochromic shifts. This combination makes them excellent candidates for the purpose of selective reporting presence of desirable molecules or media characteristics [23]. The most promising BODIPY structures are dyes with bulky aryl substituents in pyrrole rings or *meso*-position. *Meso*-aryl substituents in dipyrrin core have an ability to literally "twist", resulting in decrease in HOMO-LUMO gap; that combined with increased rigidity of the dye inhibits free rotation of aryl substituents in highly viscous media leading to decrease in non-radiative decay. This decrease in HOMO-LUMO gap combined with increased molecular rigidity leads to enhancement in fluorescence. Moreover, substituents with aromatic moieties have the ability for hydrophobic interactions and π-π staking.

Nevertheless, studies aimed at better understanding of the origin of fluorescence properties of a given probe, in particular the dependence on the environment, are scarcely addressed in the literature. That is why it remains a challenge to explore novel, simple and portable fluorescent probes for investigation of proteins. The aim of the present work was to investigate the spectral respond of several BODIPY with bulky substituents to the presence of Bovine Serum Albumin (BSA) and to propose the mechanism of the dye interaction with the protein using molecular docking.

2. Experimental part

Chemicals and reagents. Reagents were obtained from Chimmed, Aldrich, and Sigma-Aldrich Co. and used without further purification unless otherwise noted. Solvents (Chimmed, Russia) were all of analytical grade purified by standard techniques [24]. The residual water content (<0.02%) was determined by the Karl Fischer method [25]. Bovine Serum Albumin ("Agat-Med", M = 66430.3 Da) was used without further purification. All experiments were performed in phosphate buffer at pH = 7.4. For the preparation of buffer sodium dihydrogen phosphate (NaH_2PO_4, "Chimmed", Russia) and sodium hydrogen phosphate (Na_2HPO_4, "Chimmed", Russia) were recrystallized twice and dried at 110 °C then moistened with water and dried under vacuum at 36 °C for 2 days. Samples of both salts were dissolved in distilled water in quantities necessary for their solutions with a concentration of 0.2 M using literature methodics [26].

Complexes of BODIPY, differing by the nature of the substituent in the *meso*-position of the dipyrrin ligand, *4,4-difluoro-8-phenyl-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* (**1**), *4,4-difluoro-8-naphthyl-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* (**2**), *4,4-difluoro-8-antryl-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* (**3**), *4,4-difluoro-8-pyrryl-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* **(4)**, *4,4-difluoro-8-biphenyl-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* **(5)**, *4,4-difluoro-8-(4-dimethylaminophenyl)-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* **(6)**, *4,4-difluoro-8-(4-carboxyphenyl)-1,3,5,7-tetramethyl-2,6-diethyl-4-boron-3a,4a-diaza-s-indacene* **(7)** were synthesized by the methods described recently [27,28] (Fig. 1).

Fluorescence quantum yield (φ) was defined as follows:

$$\varphi_x = \varphi_{st} \cdot \left(\frac{A_x}{A_{st}}\right) \cdot \left(\frac{B_{st}}{B_x}\right) \cdot \left(\frac{n_x^2}{n_{st}^2}\right)$$

where φ_{st} is the rhodamine 6G standard quantum yield in ethanol ($\varphi = 0.95$, [29]); A_x and A_{st} are the integrated area under the corrected fluorescence spectra; B_x and B_{st} are, respectively, the absorbance (optical density) at the excitation wavelength; n_x and n_{st} are the refractive indices of solvents used for two solutions.

Radiative decay constant (k_{fl}) was calculated in accordance to equation:

$$k_{fl} = 2.9 \cdot 10^{-9} \cdot \frac{9 \cdot n^2}{(n^2+2)^2} \cdot \upsilon_{max}{}^2 \cdot \varepsilon_{max} \cdot \Delta\upsilon_{1/2}$$

where n is refractive index of solvent; υ, cm^{-1}, is wavenumber of absorption band maximum; $\Delta\upsilon_{1/2}$, cm^{-1}, is half-width of the absorption band; ε is extinction coefficient in the absorption band.

Nonradiative (k_{nr}) decay constant and fluorescence lifetime (τ) were calculated from experimentally measured fluorescence quantum yield φ and radiative decay constant k_{fl} according to the following equations:

$$\varphi = \frac{k_{fl}}{k_{fl} + k_{nr}}$$

$$\tau = \frac{1}{k_{fl} + k_{nr}}$$

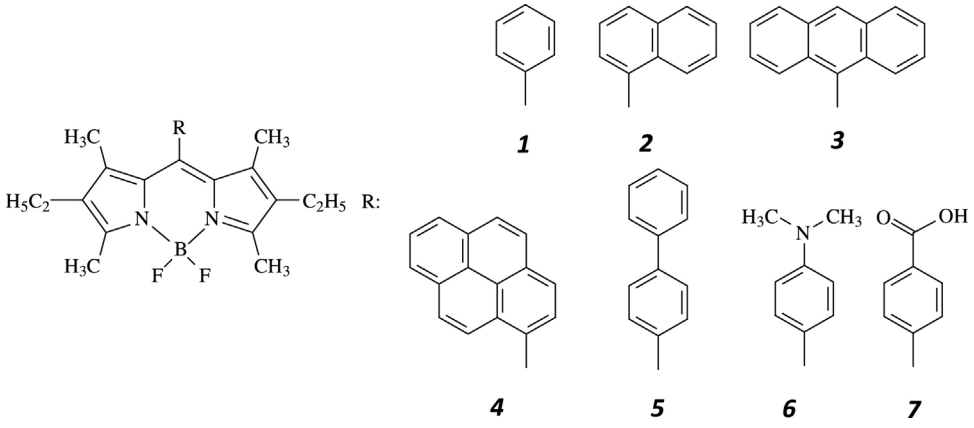

Fig. 1. Structures of investigated complexes.