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Spectroscopic study of interaction of styrylcyanine dye Sbt and its derivatives with bovine serum albumin

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ABSTRACT

The spectral-fluorescent characteristics of styrylcyanine dye Sbt ((E)-2-(4-(dimethylamino) styryl)-3-methylbenzo[d]thiazol-3-ium iodide) and homodimers, dyes conjugated with two chromophores in aqueous solutions without and in the presence of bovine serum albumin (BSA), are studied. It is established that in the presence of BSA for dyes Dbt-5 and Dbt-10, an increase of the absorptivity, a slight broadening and the emergence of new band on the short wavelength range with $\lambda_{max}=410$ nm is observed; also hypsochromic shift of the absorption and fluorescence at 30 nm and 7 nm, respectively for the dye D-183 is observed. The intensity of the fluorescence emission fundamental band in all the studied dyes in the presence of BSA increases by 3.5 to 55 times. The binding constant (K) and number of binding sites (N) of studied dyes with BSA are determined. The dependence of the binding constants with BSA from the dipole moment of dye molecules is identified, which shows that in addition to the electrostatic attraction forces between molecules of styrylcyanine dyes with BSA, hydrophobic interactions are essential. It is shown that the aggregation of dye affects the processes of interaction of the dyes with the BSA.

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1. Introduction

Organic dyes are widely used as a working medium in dye lasers [1], in analytical chemistry to determine the various trace elements [2], in photodynamic therapy [3], tissue optics [4], analysis of cells [5]etc. Depending on the sphere of application of fluorophores their various properties: quantum yield [6] and photostability [7], pH dependence [8], fluorescence lifetime [9] are essential.

In order to extend the applications the need to search for and synthesis of new organic dyes implies. In particular the search for new fluorescent probes and taps due to the increasing volume of laboratory testing and diagnosis of various diseases [10]. As fluorescent probes and markers for in vivo measurements cyanine dyes have been widely used in recent years [11,12], as well as their related class of compounds–styrylcyanine dyes [13–15]. The study of spectral-fluorescent properties of organic dyes conjugated with two chromophores — homodimers — is of great interest for various applications [16], in particular as fluorescent probes in biomedical research [17,18]. Results on the interaction between the styrylcyanine dye Sbt and its homodimers with BSA by methods of absorption and fluorescence spectroscopy are presented in this paper.

2. Materials and experimental method

Structural formulas of the studied dyes are listed in Table 1. Synthesis of the studied dyes was implemented by the Institute of Molecular Biology NAS of Ukraine, according to the methods described in [19,20].

Electronic absorption spectra were measured on a Specord 50 SA (Analytikjena, Germany) which allows measurement with an accuracy (+/−0.003 D) and resolution (0.3 nm) in the range of 190–1100 nm. Fluorescence spectra were measured on a homemade fluorescence measurement setup, assembled on the basis of monochromator MDR-12 (LOMO, Russia) with photomultiplier tube FEU-100 (Russia). High brightness LEDs were used as an excitation source. BSA ("Medpreparat", Konotop, Ukraine) was used as the protein. The bidistilled water was used as a solvent. Preparation of initial solutions of dyes was done by a volume–weight method. Working concentrations of $10^{-5}-10^{-6}$ M solutions were prepared by diluting the stock solution. The magnitude of systematic error associated with inaccurate calibration and reference distributions with different wettability of the walls of dimensional dish does not exceed 1%. The concentration of BSA (p) is defined by formula: $p=1.45\times D_{280}-0.74\times D_{260}$ (in mg/ml), where D_{280} and D_{260} — optical density of BSA solution at the wavelengths of the absorption at 280 nm and 260 nm [21]. Fluorescence titration by the method of Scatchard was carried out to determine the binding constants (K) and the number of binding sites (N) of studied dyes with BSA [22].

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Titration of the dye with BSA was carried out at constant dye concentration by serial dilution of the initial concentration of BSA. Titration of BSA and the water stain was performed at a constant concentration of BSA, by adding a sample volume of 3 ml BSA solution for 20, 40, 60, 80 and 100 μl of the initial aqueous dye. The values of K and N are determined based on the results of titrations and measurements of fluorescence spectra by the method described in [22,23]. All measurements for calculating the binding constants (K) of the dye with the protein and the number of binding sites (N) were determined by fluorescence intensity of aqueous solutions of the maximum emission band. All measurements were performed at room temperature (297 K). For ease of comparison, the presented absorption and fluorescence spectra were normalized to unity.

The calculation of the dipole moment was carried out during the quantum-chemical calculation of the structure of the dye molecules with the program package MOPAC 2009 [24] semiempirical method AM1 with a standard set of parameters [25]. Conformational search and geometry optimization of molecules were performed using restricted Hartree–Fock method and Polak–Ribier algorithm with 0.001 kcal/(Å × mole) accuracy.

3. Results and discussion

First of all, the concentration dependence of absorption and fluorescence spectra of selected dyes in water was investigated. It was established that shape of the absorption and fluorescence remains constant for all studied dyes in the concentration range $10^{-6}-10^{-5}$ M. This justifies the fact that the molecules of studied dyes are in monomeric form. The main following spectral-fluorescent characteristics of dyes in free form were determined based on experimental measurements data for aqueous solutions of the studied dyes, according to calculation procedures described in [26]: extinction coefficient (ε), oscillator strength (f_e), radiative lifetime of the excited state (τ), the frequency of purely electronic transition (ν_{0-0}) and Stokes shift (SS), which are shown in Table 2. The quantum yield of aqueous solutions of the studied dyes is relatively low — about 0.01–0.02%.

Dye Sbt is monomeric, and Dbt-5 and Dbt-10 are homodimers of dye Sbt, and their chromophores are covalently linked with each other by polymethylene chain with length $n=5$ and $n=10$, respectively. It can be seen from Table 2 that the maximum of absorption and fluorescence spectra of dyes Dbt-5 and Dbt-10 is hypsochromically shifted by 8–21 nm as compared with the maximum of the absorption spectrum and fluorescence of dye Sbt. The increase in the polymethylene chain of dyes Dbt-5 and Dbt-10 leads to decrease in the extinction coefficient by 1.9 and 6 times, respectively, as compared with the dye Sbt. Introduction of various effector groups (D-179, D-180, D-181, D-182, D-183, S-37, S-39) to the polymethylene chain leads to the fact that the absorption and fluorescence spectra is bathochromically shifted on 2–29 nm and 9–17 nm, respectively, as compared with the same spectra for the dye Dbt-10. Moreover, addition of effector groups leads to increase in extinction coefficient by 1.1–19.5 times as compared to Dbt-10.

In the presence of BSA, the shape and position of the absorption spectra of dyes Sbt, D-179, D-180, D-181, D-182, D-183, S-37 and S-39 remains constant, there is a slight decrease in the intensity of absorption by 1.3–1.5 time and bathochromic shift by 3–8 nm. In the fluorescence spectra observed increase intensity from 3.5 to 13 times and the hypsochromic shift by 3–10 nm. Adding BSA to aqueous solutions of homodimeric dyes Dbt-5 and Dbt-10 leads to an increase in absorbance and a slight broadening of the absorption spectrum. As an example, Fig. 1 shows the absorption spectra and fluorescence dye Dbt-10 by adding BSA.

In addition, a gradual increase in the amount of protein in solution at a constant concentration of dye Dbt-10 leads to the emergence of a new band on the short wavelength range with $\lambda_{max}=410$ nm. The glow intensity of fluorescence in the presence of BSA in dyes Dbt-5 and Dbt-10 increases by 54 and 55 times, respectively. The most interesting situation is observed in the absorption and fluorescence spectra in the presence of BSA of aqueous solutions of dye D-183 (Fig. 2).

The addition of BSA causes hypsochromic shift of the absorption and fluorescence spectra by 30 nm and 7 nm, respectively. The intensity of the fluorescence emission is increased by about 3 times. The increase in emission intensity of dyes Dbt-5 and Dbt-10 can be explained by the fact that their heterocycles are at a sufficient distance from each other and the trans-planar conformation is the main form of these dyes. In the presence of BSA mobility of the chromophores is reduced and becomes more rigid, which leads to increased fluorescence. Whereas the dye D-179, D-180, D-181, D-182, D-183, S-37 and S-39 implementation of various effector groups in polymethylene chain leads to a less rigid connection between the chromophores and their rotations, which disturbs the coplanarity and the emergence of the *cis-trans* isomerization. Significant conformational flexibility of molecules, which is common for aqueous solutions of dyes makes intercombination transitions with the corresponding fluorescence quenching. Cis-isomers fluoresce is usually much weaker than the *trans* isomers [27]. This difference is also enhanced by the fact that the length of the π-system along the main axis of the molecule and, consequently, the probability of electron transition in the former is much smaller than the latter. In addition, the observed phenomena can be explained by changes in the position of the ring plane dyes at their respective binding BSA. For example, if the binding of BSA molecules with the ring plane dyes are more or less parallel, that allows high coupling of π-electronic systems, the fluorescence intensity increases. On the contrary, hydrogen bonds formed in aqueous solutions of dyes lead to a perpendicular arrangement of rings and, thus, the fluorescence quenching. Broadening and increase intensity of the fundamental absorption band with $\lambda_{max}=494$ nm and the appearance of bands with $\lambda_{max}=410$ nm, the dye Dbt-10, possibly due to the formation of aggregates of molecules of this dye. It is known that one of the functions of the BSA is the transport of low molecular weight substances [28]. Depending on the content of modulating substances, the regulation of this process is carried out by changing the binding properties of albumin. The presence of molecules on the BSA-free binding sites in relation to a specific connection, as well as the extent of their binding capacity determines the functional properties of albumin in the body. A quantitative measure of the interaction of dye molecules with biomacromolecules are binding parameters: binding constant (K) and the number of binding sites (N), values are shown in Table 3.

As it can be seen from Table 3 an increase in fluorescence in 3.5 times and the binding constant of the monomer Sbt has the greatest value among all the studied dyes. At the same time, dyes Dbt-10 and Dbt-5 showed increased binding affinity to BSA. For homodimers intensity of fluorescence in the presence of BSA is 15 times greater than for the monomer Sbt. In addition, dye Dbt-10 has the highest binding constant from this series of homodimeric dyes. The same pattern was found for homodimeric dyes Dst-5, Dst-10, Tol-3, S-13 и D-184, dye-based F ((E)-4-(4-(dimethylamino)styryl)-1-methylpyridinium iodide) [15] where they showed a strong dependence of the spectral-fluorescent properties of the length of the linking group, increased affinity for binding to BSA and DNA. It should be noted that the increase in the polymethine chain in the dye Dbt-10 leads to an increase in the binding constants of 20 times, compared with the dye Dbt-5. A similar dependence was observed for dye D-181 and D-182, where the

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