Spectroscopic Investigations of Characteristics of Fluorescein's Nanomarkers in Solutions of Bovine Serum Albumin

A. A. Kuleshova, I. M. Vlasova
Lomonosov Moscow State University, Faculty of Physics, Moscow, Russia.

Abstract. This work is dedicated to investigation of the characteristics of fluorescein's nanomarkers such as the intensity of fluorescence, the degree of molecular association in the buffer solution and the changes of these characteristics in the solution of bovine serum albumin(BSA). Fluorescent nanomarkers are fluorescein and its halogens-derivatives — erythrosin, eosin and bengal rose. The characteristics of nanomarkers are changed in the solution of BSA. BSA is globular protein of blood plasma whose main function is the transport of different matter (physiological metabolites, pharmaceuticals) in blood flow. The method of fluorescent nanomarkers is used for study the physicochemical properties of binding sites of BSA. The researches of binding of fluorescent nanomarkers, such as fluorescein and its halogen-derivatives, with BSA are allowed to receive information about structure and properties of binding sites of BSA. This information has important applied medical and pharmacological aspects, as medical products binding with BSA are transported in blood flow.

Introduction

The method of fluorescent spectroscopy is practiced in the field of biomedicine, pharmaceuticals and bionanotechnology and is useful for the creation of new drugs, for the examination the influence of different pathogenic factors. The molecules and different matters can be recognized and in some cases quantitative characteristics can be found due to this method. Particularly the structure of serum albumins in the native form or ones undergoing chemical modification are investigated with the help of fluorescent spectroscopy.

The bovine serum albumin (BSA) is the globular protein of blood plasma derived from the cows (66.46 kDa, isoelectric point pI 4.9). Its concentration in plasma and serum (35–55 mg/ml) is higher than the concentrations of the other proteins. The molecules of BSA consist of 582 amino-acidic residuals. The tertiary structure is determined by three domains whose subdivide into two subdomains. The main property of molecule of BSA is the transport of physiological metabolites, it binds wide range of organic and inorganic ligands [1]. Some reactions of binding are provided by electrostatic interaction, others have covalent character, provoking chemical modifications of amino-acidic residuals' side — chains. The binding of small nanomarkers, such as fluorescein, eosin, erythrosin and bengal rose, happens to the same binding site of protein.

The luminescent markers are very sensitive even to slight changes of the complicated surrounding system (for example, living cell or buffer solution with protein). The main goal of fluorescent spectroscopy is the analysis of such kind of alterations. The method of fluorescent probes (nanomarkers) plays an important role in the investigations of physicochemical properties of binding sites of albumin [2–7]. The anionic at physiological pH nanomarkers, such as fluorescein and its halogen-derivatives, are used for researches of albumin in vitro in blood plasma [8–9].

In this work the researches of fluorescent characteristics and molecular association of fluorescein and its tetra-brominated derivative — eosin and tetra-iodinated derivative — erythrosin and tetra-chlorine-tetra-iodinated derivative — bengal rose are made and the conclusion about the binding of these nanomarkers with BSA at different values of pH is also made.

Results and Discussion

Case 1. The investigation of absorption spectra of fluorescein's family nanomarkers in the solution of BSA

The absorption spectra of fluorescein's family nanomarkers in the solution of BSA and without it were obtained at different values of pH and at various values of nanomarkers. As an example the absorption spectra of fluorescein, eosin in the solution with protein and without it is presented in Figure 1.

It is shown (Figure 1) that there is a common regularity — the position of the maximum of the nanomarkers absorption spectrum in the solutions with bovine serum albumin shifts in the red wavelength region in comparison with the solutions without BSA. Both in the solutions without protein and in BSA solutions the presence of two maximum absorption is observed (the long-wave maximum of monomers and the short-wave maximum of the nanomarkers associates).

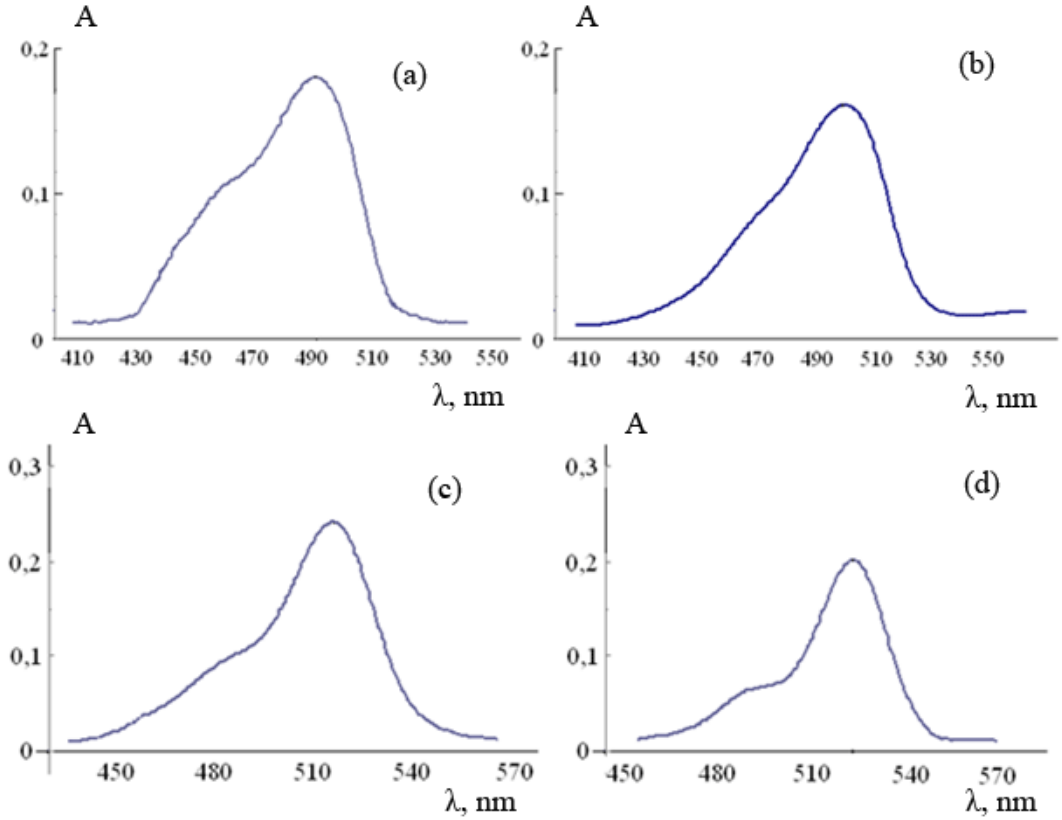

Figure 1. The absorption spectra of fluorescein in the solution without BSA (a) and with it (b) at pH 8.0. The absorption spectra of eosin in the solution without BSA (c) and with it (d) at pH 3.5.

In the work the value of nanomarkers' molecular association in the solution without and with BSA was determined at different values of pH and at various values of the nanomarkers concentration. The value of the degree of association 1-X of the solution of nanomarker is the part of the associated molecules of nanomarker 1-X in the solution, where X is the part of monomeric molecules.

In Figure 2 it is shown the dependence 1-X of fluorescein's molecules (at its different concentration) from pH in the solution without protein and with it respectively. Both in the solutions without protein and in the solutions with bovine serum albumin the dependence 1-X of fluorescein from pH has the non-linear character with the maximum at pH 6.0. The molecules of this nanomarker are electrically neutral at pH 6.0 and form the associates easy. The value of 1-X of fluorescein's molecules in the solution of bovine serum albumin is smaller than one in the solution without protein at appropriate values of pH and concentration of fluorescein. This fact is explained by the binding of the fluorescein's molecules with protein. It is shown (Figure 2) that there is the monotonous decrease of 1-X of the eosin's molecules at the increase of pH both in the solutions with BSA and in the solutions without protein. The molecules of this nanomarker are in the form of dianions at pH > 5.0 and its mutual repulsion prevents the formation of associates. The eosin's molecules lose the negative charge on COOH-group at pH from 3.0 to 5.0 and have the gently negatively charged form that simplifies the process of association. 1-X for the molecules of eosin in the solution of BSA is less than in the solution without protein at corresponding values of pH as there is the binding between the molecules of eosin with the molecules of bovine serum albumin. In Figure 3 it is shown that the monotonous decrease of 1-X of the molecules of erythrosin is observed at the increase of pH both in the solution with protein and in the solution without it, as the increase of the negative charge of the erythrosin's molecules is observed at the increase of pH. This is explained that the molecules of this nanomarker become monoanions at pH from 3.6 to 5.5 and it simplifies the process of association. 1-X of erythrosin in the solution with BSA is less than in the solution without protein at according pH, as the molecules of markers bounds with the molecules of bovine serum albumin. It is shown (Figure 3) that both in the solutions with protein and in the solutions without BSA the value 1-X of the molecules of bengal rose decreases monotonously at increasing of pH. This dependence is explained by the fact that the negative charge of bengal rose increases with the increasing of the values of pH.

The molecules of bengal rose have the dianion's form at pH > 4.0 and its mutual repulsion prevents the formation of associates. The molecules of this nanomarker become gently negatively charged monoanions at 2.6 < pH < 4.0 and it simplifies the process of association.

It is shown (Figure 3) the value 1-X of bengal rose in the solutions of BSA is less than in the ones without protein at the corresponding values of pH and the concentrations of nanomarker. This fact is explained by the bounding of bengal rose with the albumin's molecules.

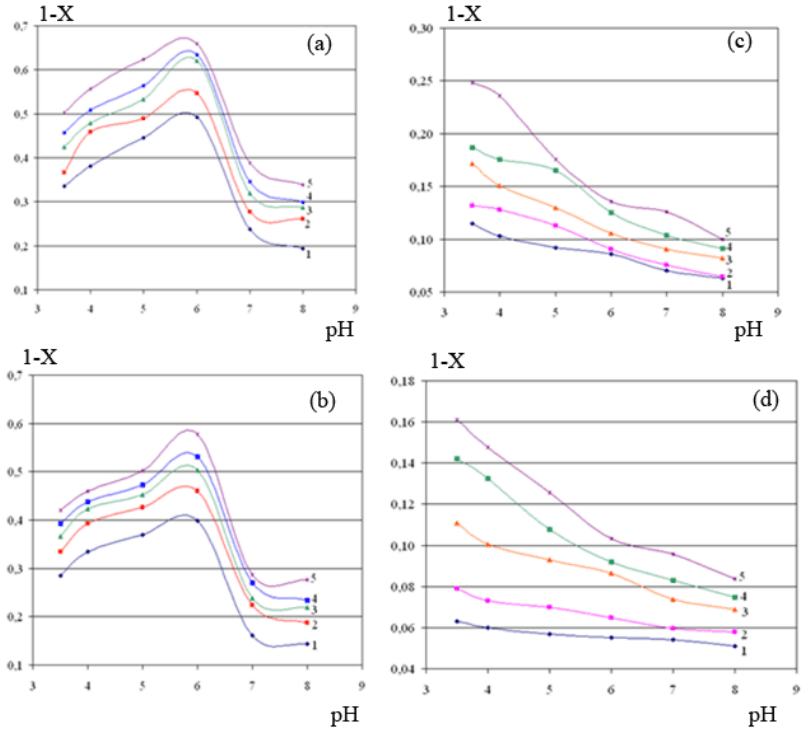

Figure 2. The degree of association of fluorescein in the solution without BSA (a) and with it (b) at different concentrations of fluorescein: 3 mkM (1); 10 mkM (2); 20 mkM (3); 30mk M (4); 50 mkM (5).
The degree of association of eosin in the solution without BSA (c) and with it (d) at different concentrations of eosin: 3 mkM (1); 10 mkM (2); 20 mkM (3); 30mk M (4); 50 mkM (5).

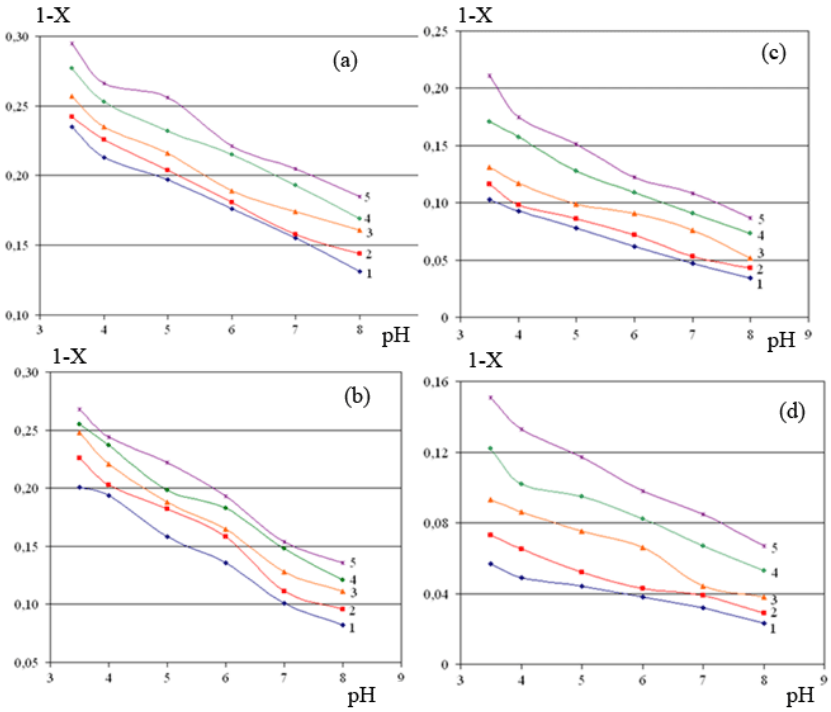

Figure 3. The degree of association of erythrosin in the solution without BSA (a) and with it (b) at different concentrations of erythrosin: 3 mkM (1); 10 mkM (2); 20 mkM (3); 30mk M (4); 50 mkM (5). The degree of association of bengal rose in the solution without BSA (c) and with it (d) at different concentrations of bengal rose: 3 mkM (1); 10 mkM (2); 20 mkM (3); 30mk M (4); 50 mkM (5).

Case 2. The fluorescence of fluorescencein's family nanomarkers in the solution of bovine albumin

The dependence of the intensity's maximum of the fluorescence spectra of fluorescein in the solution without and with BSA is represented in Figure 4. In the solution with bovine serum albumin the maximum of the fluorescein's fluorescence spectra (I_{fl}^{max}) gently moves in the red in the comparison of the solution which don't contain the protein. For example, the maximum of intensity of fluorescein's fluorescence is at the wavelength 510 nm at pH 5.0 in the solution without albumin and it is at the wavelength 515 nm at the same pH in the solution with BSA. The increase of I_{fl}^{max} of fluorescein is observed at the increasing of pH, this dependence is explained by alteration of the nanomarker's charge. The fluorescein has gently positive charge at pH < 5.5 and its possibility to fluorescence is small. In the pH value area from 5.5 to 6.8 the molecules of this nanomarker are electrically neutral. At 6.8 < pH < 8.0 fluorescein is gently negative charged and its form is monoanion. The fluorescein has a strongly negative charged form at the pH > 8.0 and has a form of dianion. The negative charged form of this nanomarker has greater intensity of fluorescence. The fluorescence of fluorescein is quenched. When this quenching is analyzed it's clear that the greater binding of nanomarker with bovine serum albumin is observed at pH 5.0–6.0, when the molecules of fluorescein have either gently positive charged form (at pH 5.0–5.5) or electrically neutral form (at pH 5.5–6.0) and the protein is gently negative charged, as the isoelectric point for bovine serum albumin is pI 4.9.

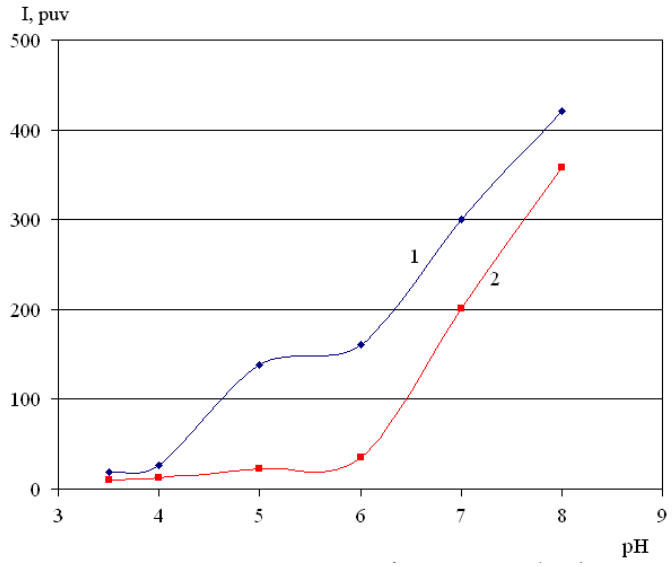

Figure 4. The dependence of the intensity's maximum of the fluorescence spectra of fluorescein in the solution without BSA (1) and with it (2).

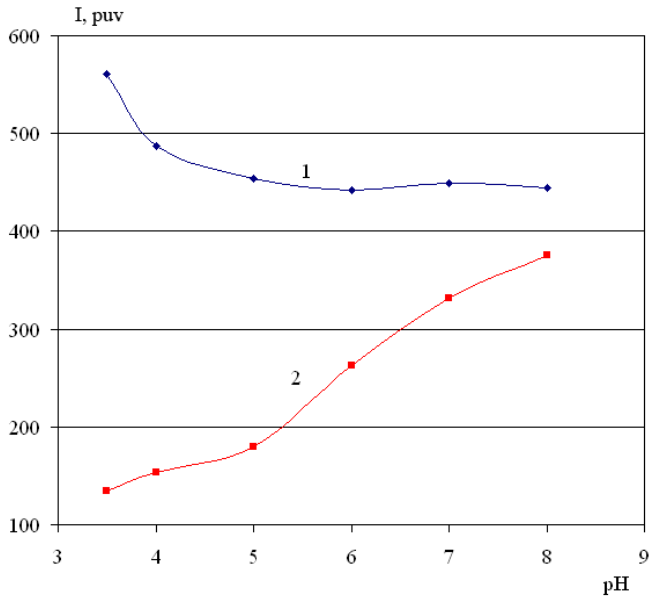

Figure 5. The dependence of the intensity's maximum of the fluorescence spectra of eosin in the solution without BSA (1) and with it (2).

There quenching of intensity and the red shift for all three nanomarker are observed in the solutions which contain the bovine serum albumin. In Figures 5, 6, 7 it is shown the dependence of intensity of nanomarkers from pH. The fluorescein's derivatives' maximum intensity decreases and then has a constant value with the increasing of pH. The molecules of nanomarkers are electrically neutral at pH < 3.0 for eosin; at pH < 3.6 for erythrosin and at pH < 2.6 for bengal rose. At 3.0 < pH < 5.0 eosin, at 3.6 <pH< 5.5 erythrosin and at 2.6 < pH < 4.0 bengal rose are gently negative charged and are gently screened by the dipole molecules of water, so the maximum value of intensity is so high. At pH > 5.0 eosin, erythrosin, at pH > 4.0 bengal rose have strongly negative charge, this leads to their strong screening by the water, therefore the value of intensity decreases. The low intensity of nanomarkers' fluorescence in the solutions with albumin (at pH < 5.0 for eosin and erythrosin and at pH > 4.0 for bengal rose) is explained by the intensive binding of fluorescence's derivatives with positive charged protein.

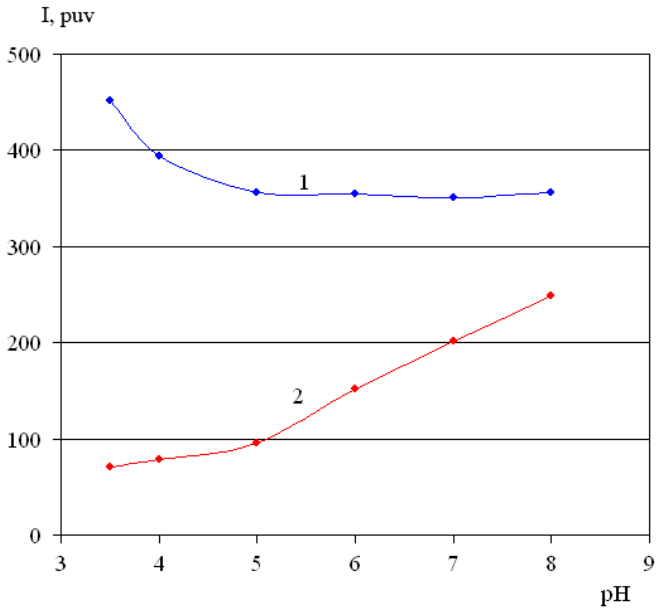

Figure 6. The dependence of the intensity's maximum of the fluorescence spectra of erythrosin in the solution without BSA (1) and with it (2).

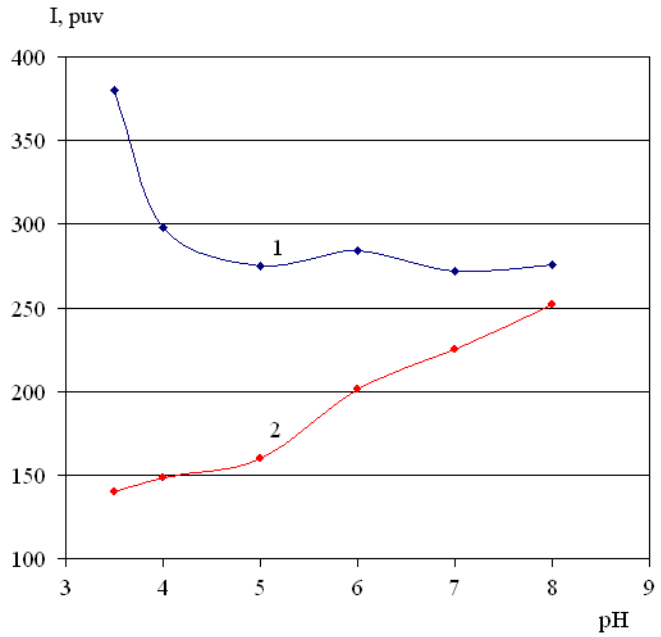

Figure 7. The dependence of the intensity's maximum of the fluorescence spectra of bengal rose in the solution without BSA (1) and with it (2).

Conclusions

The differences in the behavior of the association degree 1-X of nanomarkers were received. Fluorescein has the non-linear dependence 1-X from pH with the maximum at pH 6.0, while its halogen derivatives (eosin, erythrosin, bengal rose) have the dependence 1-X from pH which has the monotone decreased character at the increasing of pH. These differences between the association degree 1-X of fluorescein and its halogen-derivatives are explained by the value of electronegativity of lateral radicals (hydrogen of fluorescein, iodine of erythrosin, bromine of eosin, chlorine and iodine of bengal rose). The common property for all nanomarkers is the decreasing of 1-X in the solutions of BSA in comparison with the solutions without protein. In this work the analysis of fluorescence's characteristics of fluorescein and its halogen-derivatives was made at different pH in the solutions of BSA. The quenching of intensity of nanomarkers was observed in the solution of protein and the received dependences of fluorescence's intensity of nanomarkers (fluorescein, eosin, erythrosin and bengal rose) were different.

References

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