Journal of Molecular Structure 1034 (2013) 89–94

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Structure

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

Interaction of cationic surfactant cethyltrimethylammonium bromide with bovine serum albumin in dependence on pH: A study of tryptophan fluorescence

Irina M. Vlasova *, Valeria V. Zhuravleva [1], Alexander A. Vlasov [2], Alexander M. Saletsky [3]

M.V. Lomonosov Moscow State University, Physical Department, Vorob'evi Gori, 119991 Moscow, Russia

HIGHLIGHTS

- The interaction of CTAB with BSA was studied by analysis of tryptophan fluorescence.
- Denaturation of BSA at action of CTAB has one-stage mono-phase character.
- At CTAB interaction with BSA the deepest denaturation of BSA is reached at 4 mM CTAB.
- More intensive denaturation of BSA under CTAB action takes place at pH > pI of BSA.

ARTICLE INFO

Article history:
Received 29 May 2012
Received in revised form 30 August 2012
Accepted 31 August 2012
Available online 8 September 2012

Keywords:
Ionic surfactant
Bovine serum albumin
Fluorescence analysis
Tryptophan
Protein denaturation

ABSTRACT

The interaction of cationic surfactant cethyltrimethylammonium bromide (CTAB) with bovine serum albumin (BSA) at various values of pH has been studied using steady-state non-polarized tryptophan fluorescence of BSA and polarized tryptophan fluorescence of BSA. By analysis of intensity of tryptophan fluorescence of BSA, by analysis of position of maximum of spectrum of BSA tryptophan fluorescence, by analysis of polarization of BSA tryptophan fluorescence the qualitative rearrangements of BSA globules at denaturation under action of CTAB are registered. The estimation of parameters of rotational diffusion of BSA molecules helps one to determine the quantitative changes of size of BSA at CTAB-induced denaturation. It is shown that denaturation of BSA, taking place at interaction of cationic surfactant CTAB with BSA, has one-stage mono-phase character. At interaction of CTAB with BSA the deepest denaturation of BSA is reached at 4 mM CTAB (at pH 3.5–8.0). More intensive denaturation of BSA under action of CTAB takes place at values of pH, higher than the isoelectric point of BSA.

1. Introduction

The analysis of the mechanism of interaction and binding of biomolecules (such as proteins molecules) with different ligands (such as surfactants) is extremely interesting as from the point of view of a biomedicine and pharmaceuticals, so from the point of view of bionanotechnology [1–16]. The surfactants (detergents) are widely applied in biological system with protein molecules [17–30].

The bovine serum albumin (64 kDa) is the globular protein of blood plasma. Unique property of molecule of bovine serum albumin (BSA) to bind many types of organic and inorganic ligands determines one of fundamental functions of this protein – the transport of physiological metabolites and drugs. Thus the researches of the interactions of BSA with different ligands and the changes and rearrangements of BSA molecules at this interaction are extremely important.

The structural mobility of BSA molecule, provided by unique loop folding of one polypeptide chain of 582 amino-acidic residues, ensures the interaction of BSA molecule with ligands [1]. The secondary structure of BSA consists of α-helical segments and segments of random coil at physiological value of pH, the maintenance of β-sheet structures is extremely small (nearby 1%). Tertiary structure of BSA is determined by three domains.

Thus, BSA is the typical representative of homologous family of serum albumins. Levels of structural organization of BSA are almost identical to levels of the structural organization of human serum albumin (HSA) [1]. BSA and HSA are almost homologous and differ only by some amino-acidic residues. In particular, HSA contains one tryptophan residue (Trp 214), and BSA contains two tryptophan residues (Trp 135 and Trp 214).

* Corresponding author. Tel./fax: +7 495 9391489.
E-mail addresses: vlasovairina1979@mail.ru (I.M. Vlasova), Zhuravleva_V_V@mail.ru (V.V. Zhuravleva), aav1956@mail.ru (A.A. Vlasov), sam@phys.msu.ru (A.M. Saletsky).
[1] Tel.: +7 495 9391489.
[2] Tel.: +7 495 9391647.
[3] Tel./fax: +7 495 9391489.

0022-2860/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molstruc.2012.08.053

The interaction of BSA with some ligands leads to conformational rearrangements of molecules of BSA, and sometimes to denaturation of BSA. For example, among the ligands, inducing effective denaturation, are the ionic surfactants (detergents). The denaturation, as a rule, is accompanied by loss of functional properties of protein.

Surfactants represent organic amphiphilic compounds which molecules have hydrophilic segments and hydrophobic segments. By type of hydrophilic groups some types of surfactants distinguish – ionic and non-ionic. Ionic surfactants dissociate in a solution to the ions one of which are surface-active, and others (anti-ions) – are not surface-active. Depending on a sign of charge of surface-active ion the ionic surfactants distinguish to anionic, cationic and amphoteric [26].

Due to their high surface activity and ability to cause dissolution of lipids, and also due to their abilities to cause dissociation and denaturation of proteins and inactivation of viruses and toxins, the ionic surfactants are widely applied in preparation of medical pharmaceutical drugs (for example, bactericidal and disinfectant), and also are applied in dermatological and cosmetic preparations.

Among anionic surfactants, often used in medical and biochemical works to cause the protein denaturation, it is necessary to mention the sodium dodecyl sulfate (SDS). Earlier with the help of spectroscopic methods we investigated the interaction of HSA with SDS at various values of pH [22–25].

Among cationic surfactants, which can induce protein denaturation, the cethyltrimethylammonium bromide (CTAB) is often use. CTAB has bactericidal properties, and CTAB is active against Gram-positive and Gram-negative bacteria at alkaline values of pH. Earlier with the help of spectroscopic methods we investigated the interaction of HSA with CTAB at various values of pH [27–30].

It is interest to investigate BSA denaturation under action of CTAB by the analysis of intrinsic tryptophan fluorescence of BSA, both non-polarized, and polarized. Research of intrinsic tryptophan fluorescence of proteins is widely applied to an estimation of conformational state of albumin [24,25,27,29,30]. Also it is interest to determine parameters of rotational diffusion of BSA molecules in solutions with CTAB at various values of pH by researches of polarized tryptophan fluorescence of BSA taking into account Perren model of depolarization of fluorescence [31].

2. Materials and methods

2.1. Steady-state tryptophan fluorescence of BSA in solutions with CTAB

For the purpose of investigation of interaction of BSA with CTAB the following buffer solutions were prepared: 0.1 M CH_3COOH–KOH, pH (3.0–5.0) and 0.1 M KH_2PO_4–0.1 M NaOH, pH (6.0–8.0). BSA (Sigma) was diluted in buffer solutions up to concentration 5 μM.

The different concentrations of CTAB (0.5–7.0 mM) were added in BSA solutions at various values of pH (3.5–8.0).

Fluorescent investigations of samples of BSA solutions with various concentration of CTAB at various values of pH were made with the help of spectrofluorimeter LS 55 (Perkin Elmer) at room temperature. Spectra of steady-state intrinsic tryptophan fluorescence of BSA were registered in a range 300–500 nm at excitation by light with wavelength λ_{exc} = 295 nm. The obtained spectra were treated by the program FL Winlab (Perkin Elmer).

2.2. Polarized tryptophan fluorescence of BSA in solutions with CTAB

With the purpose of analysis of polarized tryptophan fluorescence of protein the BSA solutions (5 μM) with different concentrations of CTAB (0.5–7.0 mM) at various values of pH (3.5–8.0) were prepared. For the analysis of rotational diffusion of BSA molecules in total solutions the various concentration of sucrose (0–200 mM) were added.

Fluorescent investigations of samples were made with the help of spectrofluorimeter LS 55 (Perkin Elmer) at room temperature. Spectra of polarized tryptophan fluorescence of BSA were registered in a range 300–500 nm at excitation by light with wavelength λ_{exc} = 295 nm. The obtained spectra were treated by the program FL Winlab (Perkin Elmer).

The degree of polarization P of tryptophan fluorescence of BSA was calculated with the help of values of $I_{||}$ and $I_{\perp}$ in maximum of emission spectrum of protein fluorescence, where $I_{||}$ and $I_{\perp}$ – intensities of two types of fluorescence, polarized in two mutually perpendicular directions.

3. Results and discussion

3.1. Steady-state tryptophan fluorescence of BSA in solutions with CTAB

In this paragraph the investigations of interaction of CTAB with BSA molecules at various values of pH, carried with the help of analysis of steady-state tryptophan fluorescence of BSA, are presented. The spectra of steady-state non-polarized tryptophan fluorescence of BSA (λ_{exc} = 295 nm) in solutions with various CTAB concentrations at various values of pH are obtained. In Fig. 1 the dependences of intensity in maximum of spectrum (I_{fl}^{max}) of tryptophan fluorescence of BSA on CTAB concentration at various values of pH are shown. Both in the absence of CTAB, and in the presence of CTAB, at the increase of pH there is the increase of I_{fl}^{max} of tryptophan fluorescence of BSA.

At all values of pH in solutions with CTAB the quenching of tryptophan fluorescence of BSA is observed (Fig. 1), and the value of this quenching of fluorescence dependences on the value of pH. The mechanism of this is the following: the interaction of BSA with CTAB leads to denaturation of protein – to unfolding of BSA globules, to opening of hydrophobic pockets with tryptophan residues and to exposing of tryptophans (Trp 135 and Trp 214) for water molecules, which quench the tryptophan fluorescence.

It is seen (Fig. 1) that denaturation of BSA under the action of CTAB at all investigated values of pH (3.5–8.0) has one-stage character – protein globules are loosened under action of CTAB

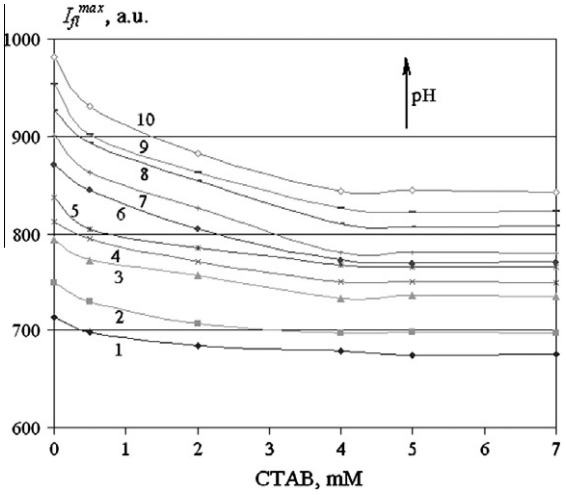

Fig. 1. The dependences of intensity in maximum of spectrum I_{fl}^{max} of tryptophan fluorescence (λ_{exc} = 295 nm) of BSA (5 μM) on CTAB concentration at various values of pH: 3.5 (1), 4.0 (2), 4.5 (3), 5.0 (4), 5.5 (5), 6.0 (6), 6.5 (7), 7.0 (8), 7.5 (9), 8.0 (10).