Process Biochemistry 49 (2014) 623–630

Contents lists available at ScienceDirect

Process Biochemistry

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

Molecular interaction of cationic gemini surfactant with bovine serum albumin: A spectroscopic and molecular docking study

Muzaffar Ul Hassan Mir, Jitendra Kumar Maurya, Shahnawaz Ali, Shah Ubaid-ullah, Abbul Bashar Khan, Rajan Patel *

Biophysical Chemistry Laboratory, Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia (A Central University), New Delhi, India

ARTICLE INFO

Article history:
Received 27 July 2013
Received in revised form
23 December 2013
Accepted 18 January 2014
Available online 27 January 2014

Keywords:
Bovine serum albumin
Gemini surfactant
Quenching
Fluorescence
Interaction
Modelling

ABSTRACT

Herein, we report the effect of *N,N′-bis(dodecyloxycarbonylmethyl)-N,N,N′,N′-tetramethyl-1,2-ethanediammonium dibromide* (dodecyl betainate gemini or DBG) on the structure and function of bovine serum albumin (BSA) by using fluorescence, time resolved fluorescence, circular dichroism and dynamic light scattering techniques. The Stern–Volmer quenching constants K_{SV} and the corresponding thermodynamic parameters viz ΔH, ΔG and ΔS have been estimated by the fluorescence quenching method. The results indicated that DBG binds spontaneously with BSA through hydrophobic interaction. Time resolved fluorescence data show that the quenching follows the static mechanism pathway. It can be seen from far-UV CD spectra that the α-helical network of BSA is disrupted and its content increases from 71% to 79% at lower concentrations which again decreases to 38% at higher concentration. DLS measurements suggested that hydrodynamic radius (R_h) decreases in the presence of 30 and 40 μM of DBG while it increases when the concentration of DBG was 70 and 100 μM. The molecular docking study indicated that DBG is embedded into subdomain IIA of BSA and binds with the R-914, R-195 and R-217 residues by hydrogen bonding and by hydrophobic interaction.

1. Introduction

Protein exhibits dualism because of the hydrophobic and hydrophilic properties of the amino acids which causes proteins to interact with amphiphilic molecules. Due to the wide variety of applications like drug delivery, cosmetics and foods, protein–surfactant interactions have become a topic of considerable interest [1,2]. Over a period of 50 years extensive studies have been done on protein–surfactant interactions [1]. Different types of physicochemical techniques have been employed to investigate the interactions between cationic and anionic surfactants with proteins in vitro [3–10]. The mechanism of such type of interactions has become an essential field of research in colloidal science [11–14]. The protein–surfactant interactions depend on many factors like charge on the head group, hydrophobic content and protein conformation [15]. In addition, the binding of surfactants to protein alters the secondary structure of proteins which affects the functional properties of proteins. It may either enhance or reduce the protein stability depending on the nature of surfactants and surfactant concentrations. Recent studies on protein–gemini systems show the alterations in the secondary structure of proteins [16–20]. Therefore, it is important to understand the protein–surfactant interactions at molecular level.

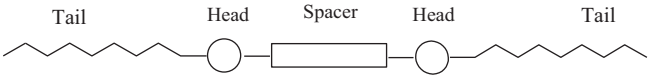
New class of surfactants referred as the second generation surfactants appeared recently in the scientific literature [21]. They bear two polar head groups and two non-polar aliphatic tails linked together covalently at or near the head groups by a third moiety called spacer, as schematically represented in Scheme 1.

Such types of surfactants are known as gemini surfactants. Due to this peculiar architecture they have properties better than those possessed by their single chain counterparts like low critical micelle concentration (cmc), low kraft temperature, strong hydrophobic microdomain [21–24]. The low cmc values of gemini surfactants lowers the concentration of free non-micellized gemini surfactant molecules which in turn reduces the toxicity of the system and enhances the ability to dissolve the water insoluble materials. Due to this, their behaviour towards proteins is quiet different than conventional surfactants. Apart from better properties, they have better applications as well. They are used as additives in hair conditioners, antiseptics, skin and eye irritation-free cosmetics, shampoos, lotions, and personal care products [25,26].

Therefore, due to these interesting and useful applications of gemini surfactants, herein, we studied the effects of DBG on BSA. The molecular interactions between DBG and BSA were observed systematically by fluorescence, time resolved fluorescence, UV–vis

* Corresponding author. Tel.: +91 8860634100.
E-mail addresses: rajanpatelpcy@gmail.com, rpatel@jmi.ac.in (R. Patel).

1359-5113/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.procbio.2014.01.020



Scheme 1. Schematically representation of gemini surfactant.

and CD spectroscopic techniques. In addition molecular docking was used to get better understanding of the interaction of DBG with BSA.

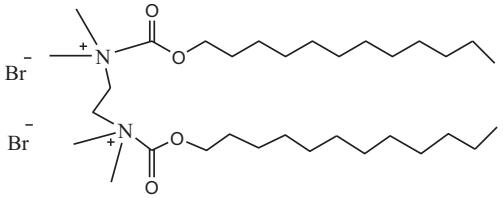
DBG is an ester based gemini surfactant with an ester linkage between the head group and the tail. Such kind of structure makes the DBG easily cleavable and easily biodegradable molecule [46], since ester linkage is a weak linkage. In other words we can say that DBG is a green molecule and it makes our system an eco-friendly. This work would contribute to a better understanding of the surfactant–protein interactions at the molecular level and also the behaviour of surfactants as denaturants. Thus, study on the ester based gemini surfactant–protein interaction is critical and undoubtedly needed.

The most abundant proteins in plasma are serum albumins. Serum albumin is synthesized in liver and exported as non-glycosylated protein [27–30]. They bind to a variety of hydrophobic ligands [4,31–33] and thus used as model proteins for many studies like biophysical, biochemical and physico-chemical [34]. Albumins play an important role in the transport and deposition of a variety of endogenous and exogenous substances in the blood [35,36] and are also used in peritoneal dialysis in fighting against the harmful effect of antibiotics [37]. Moreover, albumin is an important constituent of tissue culture media [38,39]. Studies have shown that the distribution and metabolism of a large number of biologically active compounds, such as metabolites, drugs, and even some toxins, in blood are dependent to a larger extent on their affinities towards serum albumin [40,41]. In this work we have chosen bovine serum albumin (BSA) because its structure and physiochemical properties are well characterized [42]. Moreover, the binding capacity, stability and water solubility of BSA are fairly good [43–45]. The primary structure of BSA is well known and constituted of approximately 601 amino acid residues [30] while its tertiary structure is composed of three domains and each domain containing about 190 amino acid residues giving it a molecular weight of about 66 kDa. In this work, we have discussed binding mechanism, binding properties and conformational changes induced by DBG in BSA. As per the literature survey no report has been published on the study of BSA–DBG system so far.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) was purchased from Sigma Aldrich and is used as received. Millipore water was used throughout the experiment. The gemini surfactant *N,N′-bis(dodecyloxycarbonylmethyl)-N,N,N′,N′-tetramethyl-1,2-ethanediammonium dibromide* commonly named as dodecyl betainate gemini (DBG) was synthesized in our laboratory according to the literature [46]. The structure of DBG is shown in Scheme 2. The stock solution of BSA was prepared by directly dissolving protein in phosphate buffer of pH 7.4 of 10 mM concentration. The working concentration of BSA was different in all experiments (5 μM for fluorescence, 0.25 mg/ml for circular dichroism and 2.5 mg/ml for dynamic light scattering). The concentration of BSA was confirmed by determining its absorbance at 280 nm on a Hitachi U-1500 spectrophotometer using an extinction coefficient of 43824 cm^{-1} M^{-1}.



Scheme 2. Structure of DBG.

2.2. Methods

2.2.1. Fluorescence measurements

The fluorescence spectra were recorded on a Cary eclipse spectrofluorimeter (Varian, USA) equipped with a 150 W Xenon lamp. All fluorescence spectra were collected using 1.0 cm quartz cuvette with both excitation and emission band-width of 5 nm. The intrinsic fluorescence of HSA was measured at an excitation wavelength of 280 nm with both excitation and emission band-width of 5 nm. The spectrum was recorded at three different temperature; 295, 303, and 313 K which was controlled by using constant-temperature cell holder connected to constant-temperature water circulator (Varian, USA). The synchronous fluorescence characteristics of gemini–BSA were measured from the same spectrofluorometer using different values of $\Delta\lambda$ ($\lambda_{ex} - \lambda_{em}$).

2.2.2. Time resolved fluorescence measurements

The time-resolved fluorescence measurements were performed at room temperature, using a single-photon counting spectrometer equipped with pulsed nanosecond LED excitation heads at 280 nm (Horiba, Jobin Yvon, IBH Ltd, Glasgow, UK). The fluorescence lifetime data were measured to 10,000 counts in the peak, unless otherwise indicated. The instrumental response function was recorded sequentially using a scattering solution and a time calibration of 114 ps/channel. Data were analyzed using a sum of exponentials, employing a nonlinear least squares reconvolution analysis from Horiba (Jobin Yvon, IBH Ltd), Time-resolved fluorescence decays were analyzed making use of the impulse response function (IBH DAS6 software).

2.2.3. Circular dichroism measurements

CD spectra were recorded on a Jasco-715 spectropolarimeter, equipped with a microcomputer. The instrument was calibrated with (+)-10-camphorsulfonic acid. All the CD measurements were carried out at 298 K with a thermostatically controlled cell holder attached to a Neslab RTE-110 water bath with an accuracy of ±0.1 K. Changes in the secondary structure of the protein were monitored in the far UV region (200–250 nm) using a 1 mm path length cell. The signal from the reference sample containing buffer and the detergent was subtracted from the CD signal for all measurements. To improve the signal-to-noise ratio, at least six accumulations were made for each scan. CD data were converted to concentration independent parameter, the mean residue ellipticity $[\theta]$ (deg cm^2 $dmol^{-1}$), using the relation [47]

$$[\theta]_\lambda = \frac{M_0\theta_\lambda}{10lc} \quad (1)$$

where θ_λ is the observed ellipticity in millidegrees at wavelength λ, M_0 is the mean residue weight of the protein, c is the protein concentration in grams per cubic centimetre, and l is the path-length of the cell in centimetres. The ellipticity values at 222 nm ($\theta_{222\,nm}$/mdeg) were used to calculate the α-helical content of BSA using the following equation given by Morriset et al. [48]

$$\%\alpha\text{-helix} = \frac{[\theta]_{222} + 3000}{36\,000 + 3000} \quad (2)$$

2.2.4. Dynamic light scattering measurements

DLS measurements were carried out using Laser Spectroscatter 201 (RiNA) to measure the hydrodynamic radii (R_h) of the native proteins at 298 ± 0.1 K. Protein concentration of 2.5 mg/ml was used for the measurements. All measurements were carried out at a fixed angle of 90° using an incident beam of 689 nm. The data were analyzed PMgr version 3.01 software provided by the manufacturer. For each sample 10 measurements were made with an acquisition time of 20 s. The R_h values were estimated on the basis of an autocorrelation analysis of scattered light intensity data based on translation diffusion coefficient by Stokes–Einstein relationship [49].

$$D = \frac{K_B T}{3\pi\eta d} \quad (3)$$

where k_B is Boltzmann constant, T is the temperature, η is the viscosity of water, D is the diffusion coefficient and d is diameter.

2.2.5. Molecular docking

The binding structure of DBG and 3-D structure of BSA were modelled on molecular modelling software pyMOL. Auto Dock 4 was employed to compute the possible binding mode of the DBG with BSA. The docking calculations were then performed using the Lamarckian genetic algorithm (LGA) for ligand conformational searching. Lamarckian genetic algorithm (LGA) implemented in the Auto dock was used to estimate the possible binding conformations of DBG. During the docking process, a maximum of 9 different conformations were considered for DBG. The conformer with the lowest binding free energy was used for further analysis.