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Investigating the structural integrity of Bovine serum albumin in presence of newly synthesized metallosurfactants

Preeti Garg[a], Gurpreet Kaur[a,*], Ganga Ram Chaudhary[a,*], Satwant Kaur[a], Santosh L. Gawali[b], P.A. Hassan[c]

[a] Department of Chemistry and Centre of Advanced studies in Chemistry, Panjab University, Chandigarh, India
[b] Homi Bhabha National Institute, Training School Complex, Anushakti nagar, Mumbai 400 094, India
[c] Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

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ABSTRACT

Bovine Serum Albumin is major transport protein and is often used as a drug carrier in body organs. Knowledge of its binding with metallosurfactant can significantly influence the biodistribution of metallodrugs. Current work demonstrated a facile method to prepare four different double chained metallosurfactants containing Fe, Co, Ni and Cu as part of their counter ion. The as-synthesized metallosurfactants were characterized using FTIR, AAS, TGA and XRD in solid form. The aggregation of these metallosurfactants in aqueous medium was investigated through conductivity, surface tension and SAXS. Further, we have investigated their binding with BSA through different analytical methods The effect of concentration of metallosurfactants on the primary and secondary structure of BSA was further examined by SDS-PAGE and Circular dichroism, respetively. It is found that at premicellar concentration, the primary structure of BSA was not affected but the secondary structure i.e. α-helical structure of BSA was altered as shown by circular dichroism. Interestingly, post micellar concentration of metallosurfactants shows the pronounced effect on the primary and secondary structure of BSA. SAXS study also supports the fact of unfolding of protein and its wrapping around the micelles. Zeta potential describes the electrical charge and stability of the protein in the presence of different concentration of metallosurfactant. Along with, it was found that presence of protein delays the aggregation behavior of metallosurfactant, as a sign of binding of BSA with metallosurfactant.

1. Introduction

Proteins have a property of the structural organization that fascinated the researchers over the past few decades. Among biomacromolecules, Serum albumin is one of the well explored and abundant proteins in plasma. Bovine serum albumin (BSA) has a special characteristic to display structural homology and repeating pattern of disulfides with Human serum albumin. BSA is identified as transport protein due to its stability, water solubility and versatile binding capacity to other molecules. Most of the studies had been devoted to the understanding of the molecular interaction of BSA with various surfactants, dyes, nanoparticles and polymer membranes [1–3]. Along with, protein-surfactant interactions play a major role in various applications like *in vitro* diagnostics (IVD), drug delivery, cosmetic preparation, transport of metabolites and other bioanalytical applications [4,5]. Moreover, proteins are most essential structural component of body tissues and it is important to determine the structure as well as molecular weight of proteins. Human tissue proteins' extraction and quantification is widely studied for clinical purpose e.g. in kidney biopsies. For these, a number of assays has been reported where surfactants play an important role based on the charge and size of protein due to electrostatic interaction between surfactants and proteins [6,7]. In enzyme linked immunosorbent assays, BSA is the most common blocking agent (for example ELISA applications) and inclusion of surfactant to washing buffer, removes majority of non-specific binding (including the transient binding of BSA to the surface) [8]. An et al. have studied the surfactant aided precipitation method to quantify the protein in plasma and tissues. Surfactant treatment before precipitation substantially enhanced peptide recovery and reproducibility from plasma/tissue due to denaturation or reduction of proteins by surfactants [9]. In precipitation or digestion method, surfactants enhanced the precipitation

* Corresponding authors.
E-mail addresses: gurpreet14@pu.ac.in (G. Kaur), grc22@pu.ac.in (G.R. Chaudhary).

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of BSA where cationic surfactants were valued to be superior [6]. Cloud point extraction method also used surfactants for separation of the hydrophobic membrane proteins from hydrophilic one [10]. Moreover, protein extraction was also carried out by microemulsion method where surfactant is used as a component [11]. Some more applications of surfactants and protein interactions include following examples like Liquid Crystal based sensor decorated with surfactants has also been developed to sense the proteins in mixture of serums [7]. Surfactants are also used in mass spectrometry (MS) compatible degradable surfactant (MaSDeS) for tissue proteomics that solubilizes all categories of proteins [12].

Literature reveals that study of the interaction of the proteins with cationic surfactants has been well explored. Chakraborty et al. [13] and Ghosh et al. [14] had studied the effect of hydrocarbon alkyl chain length and head group hydrophobicity on the binding affinity of BSA towards the surfactant. To modify the interaction behavior of the protein-surfactant system, Mehan et al. [15] have studied a three component system comprising of nanoparticle, BSA, and a cationic surfactant that enhanced the folding/unfolding of BSA in the free protein-surfactant system by modifying the nanoparticle-surfactant and surfactant-protein interaction.

BSA has shown a strong affinity towards the surfactant in the presence of dye. Some natural occurring amphiphiles i.e. bile salts show strong interaction with BSA labeled with fluorescein dye. This BSA-dye conjugate shows binding with bile salts but the binding of bile salts to dye-BSA was found to be weaker than the binding of bile salts to BSA [16]. Drug-protein interactions are directly linked to drug efficiency as the absorption, circulation and metabolism of drugs strongly depend on their binding properties. Metallosurfactants have been studied these days, in view of the fact that they possessed a distinct place in designing metallodrugs and alter their affinity for biomolecules. In our previous work[17], the copper metallosurfactants have been formulated that are biocompatible, cytotoxic against cancerous cells and possess antimicrobial activity [18]. In this work, attention has been paid to study the interaction of protein and metallosurfactants. A few articles were found in the literature on this aspect such as Veeralakshmi et al. [19] have evaluated nature of the interaction of the single and double chain surfactant–cobalt(III) complexes with serum albumin and observed that double chain surfactant-protein complexes are more stable as compared to the single chain surfactant-protein system due to more involvement of hydrophobic interactions. Some new magnetic surfactants had also been synthesized by mixing surfactant and polymer with gadolinium metal ions. These magnetic surfactants exhibited low cytotoxicity with a good propensity to bind with the proteins and shows a rapid protein separation in the presence of a low-strength magnetic field [20]. Yen et al. [21] have studied the binding mode of BSA with transition metal ions and demonstrated that structure of BSA remained unchanged when the binding ratio of transition metal ion to BSA was 5:1. In the above-mentioned reports, none of them clearly defined the role of the bound metal ion on the quenching, binding affinity and structural analysis of protein.

Keeping in view the importance of such study, in the present work, double chained metallosurfactants containing different transition metals (Fe, Co, Cu, Ni) were formulated via ligand insertion method having metal as a part of the counter ion. The present study also focuses on the structural properties of metallosurfactants in solid powder form and in aqueous media for their aggregation behavior. After the characterization of metallosurfactants using elemental analysis, FTIR, TGA and XRD, the critical micellar concentration (CMC) was evaluated through conductivity and surface tension. Mainly, we have investigated the BSA-metallosurfactant interaction with the four different transition metal based metallosurfactants through UV–vis spectroscopy. To analyse the structural integrity and surface charge of BSA, Polyacrylamide Gel Electrophoresis (SDS-PAGE), Circular Dichroism (CD) and Zeta Potential were carried out in presence of different concentration of metallosurfactants. SAXS was also carried out to get insight into the protein aggregation profile and structural changes produced by the interaction of metallosurfactants. This study will provide the key information on the primary and secondary structure of BSA in presence of metallosurfactants and *vis a vis* it will also evaluate the effect of BSA on metallomicelles containing Fe, Co, Ni and Cu metal ion as co-ion (as part of counter ion) of metallosurfactants.

2. Experimental section

2.1. Materials

All metal chlorides (purity ≥ 99.0%) and hexadecyltrimethylammonium chloride (CTAC) (purity ≥ 98%), Bovine Serum Albumin (BSA) (purity ≥ 98.0%, Mol. Weight = 66KDa), BSA weight marker and Coomassie Brilliant Blue were supplied by Sigma-Aldrich. Ethanol (purity ≥ 99.9%) was procured from Changshu Yangyuan Chemical China.

2.2. Synthesis of metal complexes

All metal complexes were prepared in high yield from a reaction between respective metal chloride (Fe, Co, Ni, and Cu) and CTAC in 1:2 molar ratio using ligand insertion method [17,22]. In this method, 1 g quaternary ammonium chloride and approximately 0.2 g metal chloride (Fe, Co, Ni, and Cu) were taken in 20 ml ethanol and refluxed for 2 h at 60 °C. The solvent was evaporated using rotary evaporator and metal complexes were isolated as solid powders with respective colours. Before analyses, they were recrystallized in methanol. The metallosurfactant formed were bishexadecyltrimethyl ammonium iron (II) tetrachloride; FeC II, bishexadecyltrimethyl ammonium cobalt (II) tetrachloride; CoC II, bishexadecyltrimethyl ammonium nickel (II) tetrachloride; NiC II, and bishexadecyltrimethyl ammonium copper (II) tetrachloride; CuC II.

2.2.1. Characterization

2.2.1.1. In powder form. All metal complexes were characterized by the Perkin Elmer (RX1) FTIR spectroscopy, Eager Xperience CHN under inert nitrogen medium and Phoenix-986 Atomic Absorption Spectrophotometry (AAS). XRD was carried out using Panalytical X'Pert Pro X-ray diffractometer. SDT-Q-600 (TA instruments) was used for Thermogravimetric analyses of metal complexes.

2.2.1.2. In Solution form. For CMC value, conductivity data were collected on Pico Lab India digital conductivity meter at four different temperatures (in the range of 25–40 °C) with controlled precision of ±0.01 °C. The surface tension was obtained by Du Nouy tensiometer (Kruss type 8451) at 25 °C. Two readings were taken on each sample to find out any change with time and to obtain an average value. Small-angle X-ray scattering (SAXS) experiments were performed using Anton Paar SAXSpace instrument which employs line collimated sealed tube X-ray source (Cu-Kα) operated at 40 kV, 50 mA. The scattering intensities were monitored in transmission geometry using a 2D CCD detector (pixel size 24 μm) to span a Q (momentum transfer) range of 0.01 $Å^{-1}$ to 0.65 $Å^{-1}$. The data were processed using standard protocols.

2.3. Structural analysis of BSA in presence of metallosurfactants

2.3.1. Primary and secondary structure

The analysis of the primary structure of BSA in presence of different type of metallosurfactant was carried out by SDS-PAGE using SE 260 mini-vertical gel electrophoresis unit whereas the change in