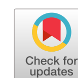
Journal of Molecular Liquids 259 (2018) 134–143

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Characterization of interactions between β-lactoglobulin with surface active ionic liquids in aqueous medium



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ARTICLE INFO

Article history:
Received 28 November 2017
Received in revised form 21 February 2018
Accepted 5 March 2018
Available online 06 March 2018

Keywords:
β-Lactoglobulin
Ionic liquids
Aggregation
Quenching
Hydrophobic interactions
Circular dichroism

ABSTRACT

The interaction of β-lactoglobulin (βLG) with surface active ionic liquids (ILs), 1-dodecyl-3-methylimidazolium bromide [C_{12}mim][Br] and 1-hexyl-3-methylimidazolium dodecylsulfate [C_6mim][$C_{12}OSO_3$] has been investigated employing tensiometry, conductivity, fluorescence, dynamic light scattering (DLS), zeta potential and circular dichroism (CD) methods. A significant difference was observed in the nature of interactions present in the βLG-[C_{12}mim][Br] system as compared to βLG-[C_6mim][$C_{12}OSO_3$]. The IL, [C_{12}mim][Br] interacted strongly with βLG to form complexes at the interface as compared to weaker interactions observed in case of βLG-[C_6mim][$C_{12}OSO_3$] system. The results of intrinsic fluorescence measurements along with CD measurements have provided insights into the unfolding of βLG due to binding of ILs. Turbidity measurements have shown the coacervate formation in case of βLG-[C_{12}mim][Br] which was also supported from DLS and zeta potential measurements. An excellent correlation of results obtained from multiple techniques has been used to characterize the interactional behavior of ILs and βLG in a wide concentration regime. The results of the present studies are expected to contribute in understanding the interactional behavior of βLG with surface active ILs and its effect on functionality of protein.

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1. Introduction

β-Lactoglobulin (βLG), a typical lipocalin, is a globular protein abundantly found in whey of ruminant species. βLG has an isoelectric point (IEP) 5.1, molecular mass of 18.5 kDa and comprises 162 aa residues, with one free cysteine residue and two disulfide bridges [1–3]. βLG exists mainly as a beta sheet protein consisting of nine anti parallel beta strands and one α-helix [4]. The native protein is composed of 10–15% of α-helix structure and 48% of antiparallel β sheet rich structure [5]. βLG exhibits complex monomer-dimer equilibria depending upon the pH of the solution and the concentration of protein [6]. The protein in its three dimensional state comprises of eight anti parallel β sheets which forms a calyx acting as binding site for most of the hydrophobic compounds. βLG is of considerable interest to the food industry because it is the major protein present in the milk whey of ruminants [7]. Another remarkable property of βLG is its ability to bind hydrophobic compounds such as retinoids, fatty acids, vitamin D, lipids and aromatic compounds [8–13].

Owing to widespread applications of surfactants in variety of biological, industrial, pharmaceutical and food processing systems, their interaction with proteins is of paramount importance [14,15]. However, the interactions between surfactants and proteins are intricate as proteins have different levels of structures. Also these interactions are dependent upon the type of surfactant, type of the protein, pH as well as temperature [16,17]. The interactions between surfactants and proteins influence the structural and morphological changes in proteins which may affect their biological activity. Upon interaction with surfactants, proteins tend to denature forming partially or fully unfolded structures with little or no tertiary structure. The rearrangement of these unfolded structures may lead to formation of different intermediate structures through aggregation or other mechanisms which play a definite role in certain human diseases [18,19]. Hence, there is growing need to understand the interactions between surfactants and proteins to control the functionality of proteins with surfactants. The interactions between βLG and conventional surfactants have been reported by several researchers. Magdassi *et al.* studied the binding of sodium dodecylsulfate (SDS) and dodecyltrimethylammonium bromide DTAB to βLG using differential scanning calorimetry, surface tension and zeta potential measurements. They evidenced the formation of surfactant/βLG complexes as well as aggregates via various interactional forces depending upon the nature of surfactant and binding ratio of surfactant to protein [20]. Taheri-Kafrani *et al.* investigated the interactions of βLG with cationic surfactant dodecyltrimethylammonium bromide (DTAB) at different pH values using spectroscopic and calorimetric techniques and concluded that DTAB induces significant alterations in its tertiary

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https://doi.org/10.1016/j.molliq.2018.03.020
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structure whereas modifies its secondary structure only slightly. However, no change in the retinol binding properties of βLG in the presence of various amounts of DTAB was observed signifying the stability of the retinol binding site [21]. In another report, the same authors reported the smaller denaturing effect of non-ionic surfactant, Triton X-100 as compared to anionic surfactant, sodium dodecylsulfate (SDS) on the tertiary structure of βLG [22]. Viseu and coworkers reported unfolding of βLG induced by cationic surfactant dodecyltrimethylammonium chloride (DTAC) and compared it with unfolding induced by chemical denaturant guanidine hydrochloride (GnHCl). By studying the kinetics of unfolding, they observed that the β to α transition in DTAC was much more cooperative as compared to the complete unfolding of protein β to D (denatured) in GnHCl [23]. The effect of various classes of surfactants (anionic, cationic and zwitterionic) on the aggregation behavior of βLG has been reported by Hansted *et al.* [24]. They concluded that the anionic and non-ionic surfactant micelles were found to inhibit aggregation of βLG by solubilizing the protein monomers thereby making them unavailable for protein-protein association. On the other hand, the cationic surfactants were found to promote the protein aggregation by combination of destabilization and charge neutralization.

In recent times, ionic liquids (ILs) have received great technological and industrial importance because of their unique properties and diverse applications [25,26]. They have also emerged as novel class of surfactants as they demonstrate superior surface activity as compared to conventional surfactants. ILs are known for solubilizing biomolecules, altering the enzyme activity and stabilization of proteins in various biomedical and pharmaceutical applications [27–29]. Therefore, it is essential to explore the interactions of surfactant like ILs with the proteins in order to optimize the technologies for their applications. There are limited reports in literature pertaining to the studies of ILs with proteins. Yu *et al.* reported that the binding of 1-tetradecyl-3-methylimidazolium bromide ([C_{14}mim][Br]) to bovine serum albumin (BSA) leads to the denaturation of protein at high [C_{14}mim][Br] concentrations [30]. The stabilization of BSA by short chain ILs, 1-alkyl-3-methylimidazolium bromide [$C_n$mim][Br] (n = 4, 6, 8) has been reported by Yan *et al.* [31]. On the other hand, Pankaj *et al.* reported significant alterations in BSA structure induced by [C_8mim][$C_{12}OSO_3$] in monomeric regimes, however, it was found to be stabilized in vesicular regimes [32]. The literature reports on studies of interaction of ILs with βLG are very few and moreover they focused on effect of short chain ILs on the native structure of βLG. The conformational transitions in βLG in the presence of ILs 1-ethyl-3-methylimidazolium ethylsulfate at different pH conditions (pH = 4 and 7.4) has been studied by Sankaranarayanan *et al.* [33]. The authors reported a sequential conversion of helical conformation to β turn and finally to β sheet structure at acidic pH due to an increase in local microviscosity. However, at neutral pH, the native β state transforms into helical state and reverts back to native β state in the presence of IL. Takeiyo and coworkers reported the IL induced structural modification of bovine milk βLG in aqueous 1-butyl-3-methylimidazolium nitrate and ethylammonium nitrate using FT-IR and circular dichroism spectroscopy [34]. They concluded that a high IL concentration leads to alterations in protein's tertiary structure by forming non-native α-helical structure of βLG.

The novel aspect of the present work lies in the fact that there are no investigations in literature dealing with the interactions between βLG and surface active imidazolium based ILs in a wide concentration range. Also, the results from the present study are expected to broaden the potential applications for SAILs and βLG. Herein, the interactions of ILs, 1-dodecyl-3-methylimidazolium bromide [C_{12}mim][Br] and 1-hexyl-3-methylimidazolium dodecylsulfate [C_6mim][$C_{12}OSO_3$] with βLG at pH 7 were investigated using various physicochemical and spectroscopic methods. The interactions of IL-βLG at the air-water interface were investigated using tensiometric measurements. A combination of techniques has been employed to resolve the interactional behavior of ILs with the βLG in broad concentration ranging from monomeric to micellar regime.

2. Experimental section

2.1. Materials

βLG was purchased from Sigma Aldrich and was used without further purification. 1-bromododecane (97%), 1-methylimidazole (≥99%), 1-hexyl-3-methylimidazolium chloride (98%), Sodium dodecylsulfate (98%) were purchased from Sigma Aldrich and used as received. AR-grade sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck, India. AR grade dichloromethane and ethyl acetate were products of S.D. Fine chem. Ltd. India. The investigated ILs were synthesized using the procedure reported elsewhere [35,36]. The purity of the compounds was checked using ^{1}H NMR. The solutions of ILs and βLG were prepared in phosphate buffer (10 mM pH = 7.4) using Sartorius analytical balance having an accuracy of ±0.0001 g. The structure of ILs and βLG has been depicted in Scheme 1.

2.2. Methods

2.2.1. Surface tension measurements

The tensiometric measurements were made on a KRUSS (Hamburg, Germany) Easy Dyne tensiometer using the ring method at 298.15 ± 0.1 K. The surface tension of doubly distilled water (71.9 ± 0.1 mN m^{-1}) was used for calibration purposes. The temperature was controlled using a thermostat and the accuracy was within ±0.1 K. All the measurements were done in triplicate with the accuracy 0.1 mN m^{-1}.

2.2.2. Conductivity measurements

The conductivity measurements were performed using Systronics 306 digital conductivity meter equipped with cell having unit cell constant. The measurements were carried out at 298.15 K and the samples were equilibrated using a thermostated glass vessel controlled by temperature controller. The conductivity meter was calibrated using freshly prepared 0.01 M AR grade potassium chloride (KCl) solution. The 0.01 M KCl solution has known conductance of 1411 μS/cm at 298.15 K. The measurements were performed with an uncertainty of <1%.

2.2.3. Fluorescence measurements

The steady state fluorescence measurements were carried out on Hitachi-4500 spectrophotometer using quartz cell of path length 1 cm at 298.15 K. Pyrene having concentration 2 μM was used as probe. The excitation wavelength used for pyrene was 334 nm and the emission spectra were recorded in wavelength range 350–500 nm. The excitation and emission slit widths were fixed at 2.5 nm. The measurements were performed in triplicate. The intrinsic fluorescence of βLG was monitored in range of 300–450 nm by providing an excitation wavelength of 280 nm.

2.2.4. Far-UV circular dichroism (CD) measurements

The circular dichroism measurements have been performed on Jasco-815 CD spectrometer at 298.15 K. The spectrum has been recorded using cuvette having path length 2 mm and the results have been expressed as average of 3 scans. The response time and the bandwidth were kept at 2 s and 0.2 nm, respectively while performing the experiments. The data is expressed as molar residue ellipticity [Θ] *vs.* wavelength (λ) given by $[\Theta] = 100\Theta_{obs}/ncl$, where Θ_{obs} is the observed ellipticity in mdeg, n is the number of amino acid residues, c is the concentration of protein and l is the path length in cm. At higher [C_{12}mim][Br] concentrations, the spectra could not be obtained with accuracy due to high HT voltage and hence the measurements were limited to lower IL concentrations only.

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