International Journal of Biological Macromolecules 83 (2016) 315–325

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Comparative insight into surfactants mediated amyloidogenesis of lysozyme

Sumit K. Chaturvedi[a], Javed M. Khan[a,b], Mohammad K. Siddiqi[a], Parvez Alam[a], Rizwan H. Khan[a,*]

[a] Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202002, India
[b] Department of Food Sciences, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form
18 November 2015
Accepted 19 November 2015
Available online 23 November 2015

Keywords:
Surfactants
pH
Lysozyme
Amyloid fibril and aggregation

ABSTRACT

Electrostatic and hydrophobic interactions have an important role in the protein aggregation. In this study, we have investigated the effect of charge and hydrophobicity of oppositely charged surfactants i.e., anionic (AOT and SDS) and cationic (CTAB and DTAB) on hen egg white lysozyme at pH 9.0 and 13.0, respectively. We have employed various methods such as turbidity measurements, Rayleigh light scattering, ThT, Congo red and ANS dye binding assays, far-UV CD, atomic force microscopy, transmission electron and fluorescence microscopy. At lower molar ratio, both anionic and cationic surfactants promote amyloid fibril formation in lysozyme at pH 9.0 and 13.0, respectively. The aggregation was proportionally increased with respect to protein concentration and hydrophobicity of surfactant. The morphology of aggregates at both the pH was fibrillar in structure, as visualized by dye binding and microscopic imaging techniques. Initially, the interaction between surfactants and lysozyme was electrostatic and then hydrophobic as investigated by ITC. This study demonstrates the crucial role of charge and hydrophobicity during amyloid fibril formation.

1. Introduction

Protein aggregation refers to an abnormal self-association of protein molecules [1]. Partial unfolding of protein results in the formation of certain critical intermediates which may rearrange by themselves to form oligomeric aggregates that are finally transformed into ordered amyloid fibrils. Protein aggregation is a part of biological functions in many organisms and also there are number of applications available in making biomaterials with potential employments [2–6]. Apart from biological functions, it also causes serious problems in medicine and industry [7]. In human, protein aggregation has been recognized as hallmark for more than 20 diseases such as Alzheimer, Parkinson, Diabetes II and many others [8–11]. Numerous proteins have been identified to form amyloid fibrils in vivo apart from differences in their amino acid sequence; this shows that amyloid formation is the intrinsic property of polypeptides but the propensity to form amyloid varies from sequence to sequence [12,13]. Several groups have demonstrated that proteins that are not associated with the disease can also form aggregate, this suggests that all proteins possess potential to aggregate but the evolutionary process cause them avoid unproductive folding pathways [14]. Moreover, protein aggregate can also be produced by exploiting different physical and chemical methods such as temperature [15], mechanical stress [16,17], freezing, thawing [18,19], cosolvent [20,21], ionic strength, pH and metal ions [22–25], etc. Amphipathic molecules like surfactants and lipids are also reported to induce aggregation in many proteins in in vitro conditions [26–29].

Hen egg white lysozyme is a small globular protein comprises of 129 amino acids, molecular weight of 14.6 kDa and its pI is around 11 [30,31]. The interior of the protein globule is almost entirely hydrophobic while the interface is covered by both the charged and non-polar amino acid residues. The considerable conformational stability is primarily attributed to the four disulfide bridges [32,33]. Lysozyme has been extensively used to understand the mechanism of protein folding, misfolding and amyloid fibril formation [34–37]. In addition, lysozyme possesses a high degree of sequence and structural homology with human lysozyme, which is associated with systemic amyloidosis in humans [38,39]. Amyloid formation by lysozyme under different conditions like high temperature, low pH, mutation and chemical modification has been previously studied [40–42]. It has also been reported that lysozyme fibrils are toxic to cell cultures [43,44]. Protein-surfactants

* Corresponding author.
E-mail addresses: rizwanhkhan@hotmail.com, rizwanhkhan1@gmail.com (R.H. Khan).

http://dx.doi.org/10.1016/j.ijbiomac.2015.11.053
0141-8130/

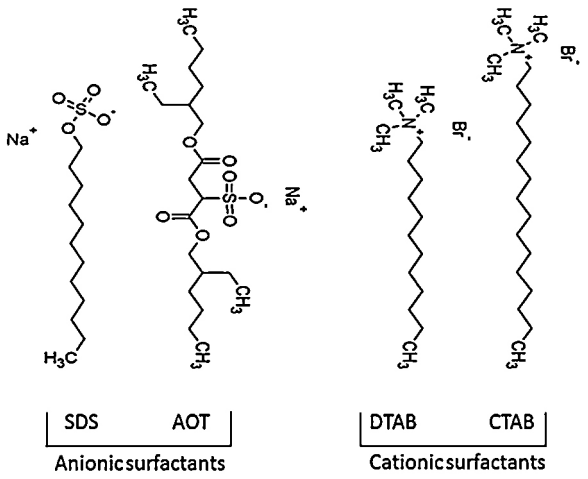

Fig. 1. Chemical structures of SDS, AOT, DTAB and CTAB.

system is known to behave as key drug delivery system and also has gained immense importance because of its relevance to the field of pharmaceutics, industries, cosmetics and biology [45–47]. Cationic surfactants act as antiseptic toward bacteria and fungi. It also has the ability to induce aggregation at lower molar ratio of surfactant to protein as exemplified by Aβ1-40 peptide and slows down the aggregation at higher molar ratio [48]. Similarly, anionic surfactants also have a great tendency to induce amyloid fibril in wide range of proteins [49,50]. Previously, our lab has reported the SDS induced aggregation in 25 diverse class of proteins when they were subjected to pH below two units of their isoelectric point (pI) while SDS was unable to induce aggregation above two units of pI [26]. But we did not check the effect of positively charged surfactants at pH above two units of pI.

In the present study, we have conducted a series of experiments to monitor the comparative effect of anionic [sodium dodecyl sulfate (SDS), sodium bisethyl sulfosuccinate (AOT)] and cationic [cetyl trimethylammonium bromide (CTAB), dodecyl trimethylammonium bromide (DTAB)] surfactants (Fig. 1) on aggregation and fibrillogenic behavior of lysozyme at pH below (9.0) and above (13.0) two units of the pI (∼11). It was tested through turbidity measurements, Rayleigh light scattering, ThT fluorescence, circular dichroism (CD), dynamic light scattering (DLS), isothermal titration calorimetry (ITC), atomic force microscopy, fluorescence microscopy and transmission electron microscopy (TEM). Since, protein–surfactant systems are extensively used as carrier for drugs and nanoparticles, it is necessary to know the nature of surfactants toward proteins. The aim of this study was to understand the role of charge, hydrophobicity of surfactants and protein concentration on the mode of aggregation. Further, this study also provides sufficient information about the comparative role of surfactants in induction as well as inhibition of protein self-assembly.

2. Materials and methods

Hen egg white lysozyme, Thioflavin T, SDS, AOT, DTAB and CTAB were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were used of analytical grade. Double distilled water was used throughout the study.

2.1. Sample preparation

A stock solution of lysozyme (500 μM) was made in 20 mM Tris–HCl buffer pH 7.4 and concentration was determined using a UV-visible spectrophotometer (Perkin Elmer Lambda 25) ε_M = 37,970 M^{-1} cm^{-1} at 280 nm. The stock of lysozyme was further diluted to attain final protein concentration to 15, 30, 50 and 100 μM in the respective buffer for the measurements and pH was further confirmed. Prior to performing the turbidity, Rayleigh light scattering, ThT, circular dichroism and TEM experiments, the protein was incubated with pH 9.0 and pH 13.0 buffer at 25 °C for 12 h followed by the addition of anionic (SDS and AOT) and cationic (CTAB and DTAB) surfactants, respectively.

2.2. pH determination

pH was measured by Mettler Toledo Seven Easy pH meter (model S20) which was routinely calibrated with standard buffers. The experiments were performed in the pH 9.0 Gly–NaOH and 13.0 KCl–NaOH buffer. All samples were filtered through 0.45 μm Millipore Millex-HV PVDF filter.

2.3. Turbidity measurement

Turbidity measurement was performed on a Perkin Elmer double beam UV-visible spectrophotometer model lambda 25 in a cuvette of 1 cm path length. The turbidity of lysozyme sample was determined by monitoring the change in optical density (O.D.) at 350 nm. Respective blank corrections were done prior to all experiments.

2.4. Rayleigh light scattering

Rayleigh light scattering measurements were taken on a Hitachi F-4500 fluorescence spectrofluorometer at the 25 °C in a cuvette of 1 cm path length. The samples were excited at 350 nm and spectra were recorded from 300 to 400 nm. Both excitation and emission slit width were set at 5 nm. The lysozyme control samples (15–100 μM) at pH 9.0 and 13 were taken to rule out the possibility of protein concentration dependent aggregation.

2.5. Thioflavin T (ThT) binding assay

A stock solution of ThT was prepared in double distilled water and filtered through 0.45 micron Millipore filter. The concentration of ThT was measured using a molar extinction coefficient ε_M = 36,000 M^{-1} cm^{-1} at 412 nm. The protein samples 15 μM, in the absence and presence of 1:10 molar ratio of protein to surfactants (anionic and cationic), were incubated at pH 9.0 and pH 13.0 for 120 min. Samples were supplemented with 15 μM of ThT solution [51]. The ThT was excited at 440 nm and spectra were recorded in the range of 450–600 nm. The excitation and emission slit widths were set at 5 nm.

2.6. Congo red (CR) binding assay

CR was prepared in double distilled water and filtered through syringe filter containing 0.45 μM membrane filter for further use. The concentration of Congo red was estimated by using ε_M 45,000 M^{-1} cm^{-1} at 498 nm. Aliquots containing CR (15 μM) with protein (15 μM) in the absence and presence of 1:10 molar ratio of protein to surfactants (anionic and cationic) were taken and kept for 15 min at 25 °C [52]. The absorbance spectra (400–700 nm) were recorded with a UV-visible spectrophotometer (Perkin Elmer Lambda 25) in a 1 cm path length cuvette.

2.7. ANS fluorescence measurements

The steady-state fluorescence measurements were performed on Schimadzu spectrophotometer (RF-5301 PC). Both excitation and emission slits were set at 5 nm. For ANS binding experiment, protein samples (incubated for 120 min at 25 °C) at pH 9.0 and pH