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Effect of surfactants on Ra-sHSPI – A small heat shock protein from the cattle tick *Rhipicephalus annulatus*

Mohammad Khursheed Siddiqi [a], Yasser E. Shahein [b], Nahla Hussein [b], Rizwan H. Khan [a, *]

[a] Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202002, India
[b] Molecular Biology Department, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Cairo, Egypt

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ABSTRACT

Electrostatic interaction plays an important role in protein aggregation phenomenon. In this study, we have checked the effect of anionic – Sodium Dodecyl Sulfate (SDS) and cationic-Cetyltrimethyl Ammonium Bromide (CTAB) surfactant on aggregation behavior of Ra-sHSPI, a small heat shock protein purified from *Rhipicephalus annulatus* tick. To monitor the effect of these surfactants, we have employed several spectroscopic methods such as Rayleigh light scattering measurements, ANS (8-Anilinonaphthalene-1-sulfonic acid) fluorescence measurements, ThT (Thioflavin T) binding assays, Far-UV CD (Circular Dichroism) and dynamic light scattering measurements. In the presence of anionic surfactant-SDS, Ra-sHSPI forms amyloid fibrils, in contrast, no amyloid formation was observed in presence of cationic surfactant at low pH. Enhancement of ANS fluorescence intensity confirms the exposition of more hydrophobic patches during aggregation. ThT binding assay confirms the amyloid fibrillar nature of the SDS induced Ra-sHSPI aggregates and supported by PASTA 2.0 (prediction of amyloid structural aggregation) software. This study demonstrates the crucial role of charge during amyloid fibril formation at low pH in Ra-sHSPI.

1. Introduction

Protein molecules tend to self-associate to form aggregates of larger size. Partial unfolding or conformational alteration to the protein is usually believed to be a prerequisite condition for aggregate formation [1]. Deposition of these protein aggregates in the cell (intracellularly or extracellularly), remarkably, as amyloid aggregates associated with various pathophysiological conditions such as Alzheimer's disease [2], Parkinson's disease [3], and spongiform encephalopathy [4]. Amyloid fibrils are ordered protein aggregates enriched with cross β-sheet structures. They are highly toxic and their accumulation in the neuron cells leads to its degeneration; affected person suffered with dementia (e.g., Alzheimer's disease) [5]. Apart from harmful effects of protein aggregates, it also performs several crucial function in living organisms [6]. Moreover, proteins that are not associated with amyloidoses also tend to form amyloid aggregates. This inferred that amyloid formation is an intrinsic property of the polypeptide chain but propensity to form amyloid aggregate depends on polypeptide sequence [7]. Moreover, protein aggregation can also be induced by exploiting different physical and chemical methods like temperature [8], cosolvent [9] etc. Amphipathic molecules also emerged as contributor of protein aggregate formation in many proteins [10–16].

Small heat shock proteins are proteins having low molecular mass (ranging from 12 to 43 kDa) [17]. They come under the category of ATP independent chaperones and functionally active in their oligomeric state. Amino acid sequence analysis shows significant similarity with the alpha crystalline vertebrate eye lens. N terminal end is usually characterized by the presence of alpha crystalline domain and evolutionally conserved [18,19] whereas C terminal domain is poorly conserved [17,20]. Small heat shock proteins are able to tolerate lethal thermal stress condition and reported to play important role in malaria [21] and filaria [22]. In ticks, these proteins are expressed during blood feeding [23] and infection [24].

Surfactants are amphipathic molecules which have a charged head group and a long aliphatic chain [25]. Anionic surfactant – SDS, has negatively charged head group [26–29] whereas cationic surfactant – CTAB, has positively charged head group [30–32].

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

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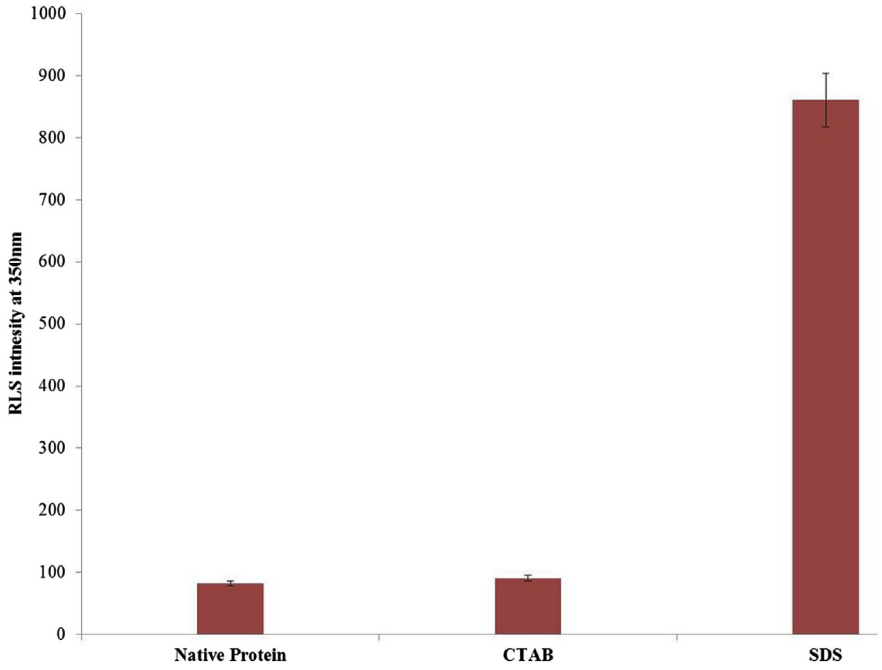

Fig. 1. Rayleigh light scattering (RLS) of Ra-sHSPI (50 μg/ml) at 350 nm in the absence and presence of 5 mM of CTAB and SDS at pH 2.0.

Since, it mimics the phospholipids present in the biological membrane, it carries a great hope for understanding protein and phospholipid interaction. In consistence, phospholipids were reported to induce the formation of amyloid aggregates [33]. Furthermore, our lab have also reported that anionic surfactant induces the amyloid fibril formation [10].

In this study, we investigated the effect of surfactant on small heat shock protein from *Rhipicephalus annulatus* (Ra-sHSPI) by exploiting different spectroscopic techniques like fluorescence spectroscopy, circular dichroism and dynamic light scattering measurement.

2. Materials and methods

Small heat shock protein was purified from *Rhipicephalus annulatus*; Thioflavin T, SDS and CTAB were procured from Sigma Aldrich Chemical Co. India. Double distilled water was used throughout the experimental study. All other reagents were used of analytical grade.

2.1. Purification of protein

Rhipicephalus annulatus small heat shock protein was expressed and purified under native conditions according to the methodology discussed in our previous paper [34]. The isoelectric point of protein was 7.76.

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 at pH 2.0 in the 20 mM Gly-HCl buffer. All samples were filtered through 0.45 μm Millipore Millex-HV PVDF filter.

2.3. Rayleigh light scattering

Rayleigh light scattering measurements were taken on a Hitachi F-4500 fluorescence spectrofluorometer at 25 °C in a cuvette of 1 cm path length. Protein samples of 50 μg/ml, in the absence and presence of 5 mM of surfactants (anionic and cationic), were incubated at pH 2.0 for overnight. The isoelectric point of protein was 7.76. The samples were excited at 350 nm and spectra were recorded from 300 to 400 nm. Both excitation and emission slit widths were set at 5 nm. The Ra-sHSPI control samples (50 μg/ml) at pH 2 were taken to rule out the possibility of protein concentration dependent aggregation. To rule out the possibility of scattering by surfactant itself, respective blanks were subtracted.

2.4. ANS fluorescence measurements

The steady-state fluorescence measurements were performed on Shimadzu spectrofluorophotometer (RF-5301 PC). Both excitation and emission slits were set at 5 nm. For ANS binding experiment, protein samples (incubated for overnight at room temperature) at pH 2.0 were incubated with 50 fold molar excess of ANS for 30 min at 25 °C in dark. The excitation wavelength for ANS fluorescence was set at 380 nm and the emission spectra were recorded from 400 to 600 nm. The protein concentration was fixed at 50 μg/ml and concentration of surfactants was 5 mM (for both anionic and cationic surfactants).

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 $\varepsilon_M = 36000\ M^{-1}cm^{-1}$ at 412 nm. The protein samples of 50 μg/ml, in the absence and presence of 5 mM of surfactants (anionic and cationic), were incubated at pH 2.0 for overnight. Post incubation, samples were supplemented with 15 μM of ThT solution, and were further incubated for 30 min in the dark. 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. Appropriate blank corrections were done prior to all measurements. Prior to all measurements all samples were incubated at 25 °C for 12 h.