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Research Letter

Use of Viscosity to Probe the Interaction of Anionic Surfactants with a Coagulant Protein from *Moringa oleifera* Seeds

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The intrinsic viscosity of the coagulant protein was evaluated from the flow times of the protein solutions through a capillary viscometer, and the results suggested the coagulant protein to be globular. The interactions of the coagulant protein with anionic surfactant sodium dodecyl sulphate (SDS) and sodium dodecyl benzene sulfonate (SDBS) were also investigated by capillary viscometry. We conclude that there is strong protein-surfactant interaction at very low surfactant concentrations, and the behavior of the anionic surfactants in solutions containing coagulant protein is very similar. The viscometry results of protein-SDS system are compared with surface tension, fluorescence, and circular dichroism reported earlier. Combining the results of the four studies, the four approaches seem to confirm the same picture of the coagulant protein-SDS interaction. All the physical quantities when studied as function of surfactant concentration for 0.05% (w/v) protein solution either exhibited a maximum or minimum at a critical SDS concentration.

1. Introduction

Moringa oleifera is a multipurpose tropical tree with most of its parts being useful for medicinal and commercial applications in addition to its nutritional value [1–8]. The seeds from this plant contain active coagulating agents characterized as dimeric cationic proteins having a molecular weight of 13 kDa and an isoelectric pH between 10 and 11 [2]. It is suggested that in the near future, coagulating protein extracted from *Moringa oleifera* seeds could be a potential challenger of synthetic coagulants (e.g., iron chloride and aluminium sulphate) for water purification [3] and for primary treatment of industrial and domestic wastewaters [9]. As a result, it has been recommended by the Food and Agricultural Organization (FAO) as a proper and advisable way for treating water [8]. Recently, protein extract from *Moringa oleifera* seeds has been found to be an anionic surfactant-removal agent in aqueous solutions [4].Thus, it is important to keep on researching on protein extracts from *Moringa*'s properties.

The interaction between surfactants and proteins is an active field of research in colloid science because very often in practice the proteins and surfactants are present in the same systems such as industrial, biological, pharmaceutical, and cosmetic systems. In addition, physicochemical characterisation of seed proteins is important in order to understand the nature of the protein and its interaction with other components.

It is known in general that anionic surfactants interact strongly with proteins and form protein-surfactant complexes, which would induce the unfolding of proteins [10]. The sensitivity of viscosity to molecular structure makes it useful for monitoring processes that result in changes in overall protein conformation [11, 12]. Apart from viscosity measurements, other classical techniques such as surface tension and fluorescence spectroscopy have been employed to examine the occurrence of critical phenomena in solution properties of protein-surfactant mixtures [13, 14]. As has been established using surface tensiometry and intrinsic fluorescence spectroscopy, the anionic surfactant sodium dodecyl sulphate (SDS) interacts strongly with the coagulant protein from *Moringa oleifera* seeds [15]. Similar interactions were observed between the coagulant protein and the anionic surfactant sodium bis(2-ethyl-1-hexyl)sulfosuccinate (AOT) when monitored by surface tension probe [16].

In this work, the effects of the presence of anionic surfactants SDS and SDBS on the viscosity properties of the coagulant protein from *Moringa oleifera* are reported. The results of solution properties of the protein in the presence of SDS are verified by surface tension, circular dichroism, and fluorescence measurements reported earlier [15–18].

2. Materials and Methods

2.1. Extraction and Purification. The extraction and purification of protein powder was done using the method of Ndabigengesere and Narasiah [3], and the experimental details are described in our previous work [15–18].

2.2. Viscosity and Protein-Surfactant Interactions Measurements. The viscosity measurements were carried out using an Ubbelohde suspended-level capillary viscometer. The viscometer was suspended vertically in a thermostat water bath at 25 ± 0.1°C. The solutions were allowed to equilibrate for 10 minutes before beginning timed runs. The flow time of a constant volume of solution through the capillary was measured with a digital stopwatch.

The intrinsic viscosity $[\eta]$ of the coagulant protein was measured for protein concentration, c, in the range 0.02–0.15 g/mL in 0.1 M NaCl. Solution environment such as presence of surfactants can affect protein conformation. To study interactions SDS and SDBS with the coagulant protein, capillary viscosities of the surfactant/protein solutions were measured using a capillary viscometer. The protein concentration (% w/v) was kept constant at 0.05% whereas the surfactant concentration was varied up to concentrations higher than the critical micelle concentration (*CMC*). The protein solution was used as the reference standard for surfactant dissolved in 0.05% protein. The SDS (99% purity) was supplied by Sigma-Aldrich whereas SDBS was supplied by Fluka, and both surfactants were used without further purification. The measurements of surface tension, fluorescence, and circular dichroism spectral correlation coefficients of SDS solutions in the presence of protein were done in similar manner, and the details are described elsewhere [15–18].

3. Results and Discussion

3.1. Intrinsic Viscosity. For background ionic strength capable of swamping contributions to the ionic strength from the protein, the Huggins and Kraemer equations, respectively, are

$$\frac{\eta_{sp}}{c} = [\eta]_H + k_H[\eta]_H^2 c,$$
$$\ln\frac{\eta_r}{c} = [\eta]_K + k_K[\eta]_K^2 c, \quad (1)$$

where η_r is the relative viscosity, η_{sp} $(= \eta_r - 1)$ is the specific viscosity, c is the protein concentration, and k_H and k_K are the Huggins and Kraemer's constants, respectively, [19–21]. η_{sp} is dependent on concentration and interaction forces. Figure 1 shows the plots obtained by fitting the experimental data to (1). The plots are linear with statistical correlation coefficient, R^2, of 0.999 and 0.995 for Huggins plot and Kraemer plot, respectively. Thus, the $[\eta]$ was estimated from the intercepts, giving $[\eta]_H = 2.4$ mL/g and $[\eta]_K = 2.6$ mL/g. Thus, $[\eta]$ of the coagulant protein was estimated as the average of the two values is 2.5 mL/g. Very high protein concentrations (up to 0.15 g/mL) were used for intrinsic viscosity determination. If the polymer concentration is very high while the intrinsic viscosity is very low, the molecule must be globular with a compact, nearly spherical shape [12]. The $[\eta]$ has been defined as the shape factor, and a value between 2.5 and 4 mL/g indicates globular particles [22].

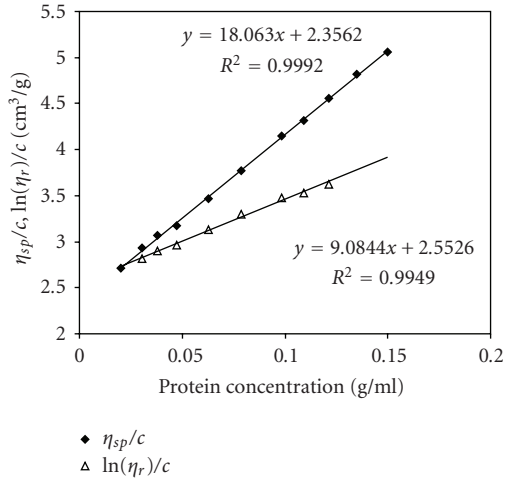

Figure 1: Evaluation of intrinsic viscosity for solutions in 0.1 M NaCl of the coagulant protein at 25°C. The Huggins (filled diamonds) and Kraemer (open triangles) plot give $[\eta]_H = 2.4$ mL/g and $[\eta]_K = 2.6$ mL/g, respectively.

3.2. Protein-Surfactant Interaction. Viscometry is effective in probing conformational changes due to interaction of protein with ionic surfactants [23]. The measurement of solution viscosity was performed to monitor the interaction of the coagulant protein with anionic surfactants SDS and SDBS in water. Results in Figure 2 show that addition of the anionic surfactants to the protein solutions gives a strong initial increase in viscosity and then a decrease, through a maximum at 0.768 mM surfactant concentration. The maximum suggests a change in the conformation of the protein structure or aggregation [24]. Upon further increase in the surfactant concentration above 2 mM, the viscosity increases gradually (Figure 2), suggesting protein restructuring and a significant expansion of the protein molecule coil in solution [24]. The behavior of the two anionic surfactants in solutions containing coagulant protein is very similar.

It is not possible from viscosity measurements alone to establish the origin of the viscosity changes that occur when surfactants are added to the coagulant protein solutions. A variety of physicochemical phenomena may contribute to the overall viscosity changes, including surfactant binding, protein aggregation, protein conformational changes, and

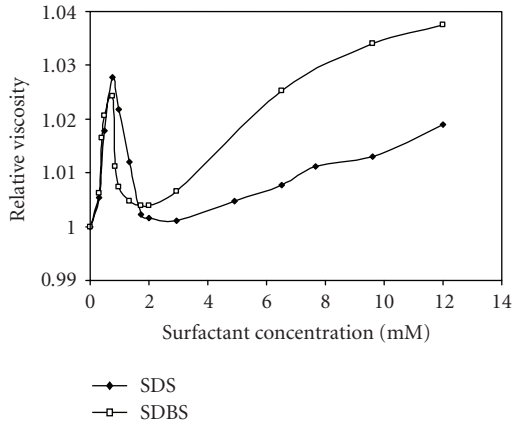

Figure 2: Influence of anionic surfactants on the relative viscosity of solutions containing 0.05% of the coagulant protein in water at 25°C.

surfactant micellization. The large increase in viscosity at low surfactant concentration may indicate the formation of aggregates. The coagulant protein is highly cationic in water (i.e., in the absence of surfactants) because the protein is below its isoelectric point [2]. Consequently, there is an electrostatic repulsion between the protein molecules that is large enough to prevent them from associating [25]. Thus, at a very low surfactant concentration, there should be strong electrostatic interactions between the negatively charged surfactants and the positively charged groups in the coagulant protein. This reduces the net positive charge on the protein, and eventually, electrical net charge would no longer be large enough to prevent the protein-surfactant complexes formed from aggregating [26]. This is the surfactant concentration where the electrical charge on the protein molecules is close to zero, that is, charge neutralization occurs [26]. Further binding of anionic surfactant to the protein molecules caused them to gain a net negative charge sufficiently large such that electrostatic repulsions between the protein-surfactant molecules oppose aggregation, and so the aggregates would dissolve [25], which could explain why the solution viscosity decreases again (Figure 2).

3.3. Combined Analysis of the Coagulant Protein-SDS Interaction. In this section, the viscosity data was directly compared to earlier reported surface tension [15, 16], fluorescence [17], and CD [18] data of the coagulant protein-SDS interaction studies (Figure 3). Similar SDS concentration ranges were used, and it was convenient to divide the profiles into four different regions depending on the SDS concentration.

The main point is that in region I, (<2 mM SDS), the bulk solution and interfacial properties exhibit critical behavior (marked by the arrows in Figure 3). It can be seen that the minimum in the spectral correlation coefficients and maxima in the relative viscosity, fluorescence intensity, and surface tension occurs at approximately the same concentration of SDS of about 1 mM. The authors suggest that this marks the stoichiometric balance point, where charge neutralization between the negatively charged SDS

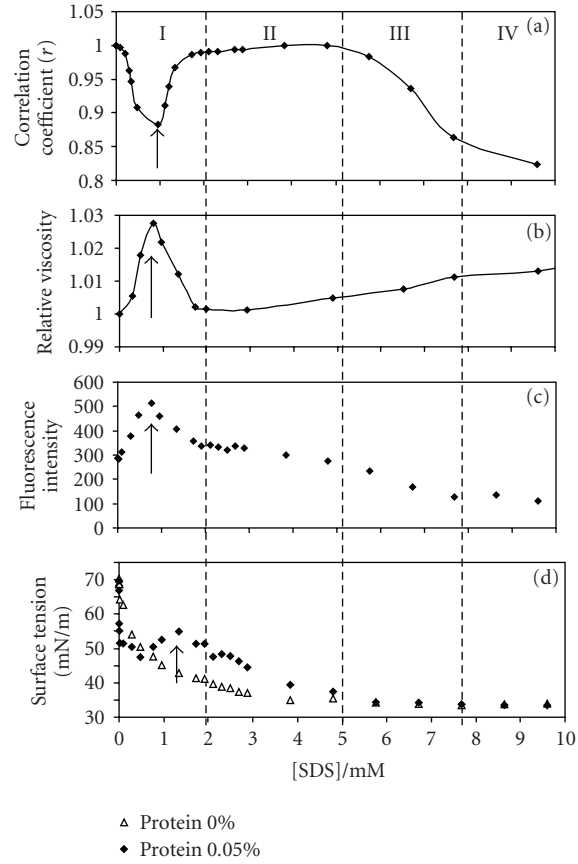

Figure 3: Variation with SDS concentration of (a) spectral correlation coefficient, (b) relative viscosity, (c) protein fluorescence intensity and (d) surface tension in the presence of protein. The protein concentration was 0.05% w/v.

and the positively charged fragments in the coagulant protein occur [27–29]. Below 1 mM SDS, it is suggested that the coagulant protein-SDS complexes form aggregates.

Although charge neutralization and aggregation of protein/surfactant mixtures has been reported before [25, 26, 30, 31], the conclusion that the SDS bound to the coagulant protein promotes protein aggregation ([SDS] <1 mM) and opposes protein aggregation (1 mM < [SDS] <2 mM) is presently not well understood. But it does provide the framework for testable models. These issues are being addressed in our on-going research work.

4. Conclusions

The determined intrinsic viscosity of the coagulant protein is 2.5 mL/g. The intrinsic viscosity value suggests globular nature. The interaction between the coagulant protein and anionic surfactants, SDS and SDBS, in aqueous medium was investigated by viscometry and compared with tensiometry, fluorescence, and CD results previously reported. The occurrence of critical phenomena in solution properties of mixed coagulant protein-anionic surfactants system has been observed. Put together, these four approaches seem

to confirm the same picture of the coagulant protein-SDS interaction.

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