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Effect of galactose on acid induced molten globule state of Soybean Agglutinin: Biophysical approach

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ABSTRACT

In the present study the formation of molten globule-like unfolding intermediate Soybean Agglutinin (SBA) in acidic pH range has been established with the help of acrylamide quenching, intrinsic fluorescence, ANS fluorescence measurement, far UV CD and dynamic light scattering measurement. A marked increase in ANS fluorescence was observed at pH 2.2. Ksv of acrylamide quenching was found to be higher at pH 2.2 than that of native SBA at pH 7. Far UV CD spectra of pH induced state suggest that SBA shows significant retention of secondary structure closure to native. Hydrodynamic radius of SBA at pH 2.2 was found be more as compared to native state and also in other pH induced states. Further we checked the effect of galactose on the molten globule state of SBA. This study suggests that SBA exist as molten globule at pH 2.2 and this study will help in acid induced molten globule state of other proteins.

1. Introduction

The mechanism by which protein fold to their unique biologically active state is a very intricate process. The folding process is even more complicated in multimeric proteins where each subunit may fold independently and inter subunit interactions may also affect the folding process [1]. The elucidation of molecular mechanism of protein folding remains one of the major challenges in biochemistry [2]. The study of molten globule state helps us to understand the role of various forces that comes into play during folding process of proteins [3,4]. The molten globule is an intermediate between native and denatured state which retains significant amount of secondary structure but there exist disorderness in the tertiary structure of the protein [5]. The molten globule state under low or high pH or in low concentration of denaturants such as urea and guanidine-hydrochloride (GuHCl) has been observed previously for many proteins [6]. Molten globule state generally corresponds to late folding intermediates of proteins which are difficult to attain at neutral pH. Hence for studying molten globule state extremes of pH, co-solvents and denaturants are generally used [7,8].

Lectins are proteins or glycoproteins' that precipitate complex carbohydrates or polysaccharides agglutinate cells. Moreover, their interaction resembles antigen–antibody or enzyme substrate reactions. Due to their high degree of specificity with carbohydrates, lectins are useful model for protein-carbohydrate interactions. Soybean agglutinin belongs to family of legume lectins and is an oligomeric beta sheet protein and it contains six tryptophan residues per subunit [9]. The legume lectins are among the most extensively studied family of proteins and there is high degree of homology in their tertiary structure. SBA exists as a tetramer in its native state which is very similar to Con A lectin which is described as jelly roll motif that comprises three antiparallel beta sheets: a six-stranded flat 'back', a seven-stranded curved 'front', and a five-stranded 'top' that forms a roof-like structure above the other two [10]. The three dimensional structure of SBA has been determined by X-ray diffraction analysis [11].

Previously our group has reported the folding and molten globule states of many proteins [12–17]. In this work we report pH dependent states of Soybean lectin. Changes in secondary as well as tertiary structure were monitored by far UV-CD measurements. Furthermore, we investigate ANS (8-Anilino-1-naphthale nesulfonic acid) binding a hydrophobic patches specific probe using fluorescence measurements. We also monitor the effect of galactose on the molten globule state of SBA.

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2. Material and methods

2.1. Materials

Soybean lectin, 1-anilinonaphthalene- 8-sulfonate (ANS), 4-p-nitrophenyl butyrate, acrylamide, galactose and guanidine-hydrochloride (GuHCl) were purchased from Sigma Chemical Co. St. Louis, MO. All other reagents used in the study were of analytical grade.

2.2. Methods

2.2.1. Protein concentration determination

Protein concentration was determined from the specific extinction coefficient of $A^{1\%}_{280}$ ~ 12.8 for SBA or alternatively by method of Lowry et al. [18]. All samples were filtered through 0.45 μm filters.

2.2.2. pH and galactose treatment of Soybean lectin

pH measurements were carried out on Mettler Toledo pH meter (Seven Easy S20-K) model using an Expert "Pro3 in 1 type electrode. The least count of the pH meter was 0.01 pH unit. The pH meter was routinely calibrated at room temperature with either 0.05 M potassium hydrogen phthalate buffer, pH 4.0 in the acidic range or 0.01 M tetra borate buffer, pH 9.2 in the alkaline range. pH-induced unfolding of Soybean lectin was carried out in 20 mM of the following buffers: pH 1–1.6 (KCl–HCl buffer), pH 1.8–3.0 (Gly–HCl buffer), pH 3.5–5.0 (Na–acetate buffer), pH 6.0 (Sodium phosphate buffer), pH 7. (tris–HCl buffer). Protein was incubated for 12 h at room temperature in different pH before spectroscopic measurements were recorded. In order to check the effect of galactose, samples were incubated with 50 mM concentration of galactose for overnight in order to monitor its influence on the molten globule state unless otherwise mentioned.

2.2.3. Fluorescence measurements

Fluorescence measurements were performed on a Shimadzu spectrofluorimeter, model RF-540 equipped with a data recorder DR-3. The fluorescence spectra were measured at 25 ± 0.1 °C with a 1 cm path length cell. Protein concentration was taken as 5 μM and the excitation and emission slits were set at 5 and 10 nm, respectively. A stock solution of ANS was prepared in distilled water and its concentration was determined using an extinction coefficient of $\varepsilon_M = 5000\ M^{-1}\ cm^{-1}$ at 350 nm. 1:20 M ratio of protein to ANS was taken. For ANS fluorescence in the ANS binding experiments, the excitation was set at 380 nm and the emission spectra were recorded in the range of 400–600 nm.

2.2.4. Acrylamide quenching experiments

Fluorescence quenching experiments were carried out by adding small aliquots of (5 M stock) of acrylamide to protein solution (5 μM). The protein samples were excited at 295 nm and spectra were recorded in the 300–400 nm range. The excitation and emission slit width were 5 and 10 nm, respectively. Quenching data were analyzed according to Stern–Volmer equation

$$F_o/F = 1 + K_{sv}[Q] \quad (1)$$

where F_o and F are the fluorescence intensities in absence and presence of quencher (acrylamide), [Q] is the molar concentration of the quencher and Ksv is the Stern–Volmer quenching constant [19].

2.2.5. Circular Dichroism (CD) measurements

CD measurements were carried out with a Jasco Spectropolarimeter, model J-720 equipped with a microcomputer. The instrument was calibrated with d-10-camphorsulphonic acid. All CD measurements were performed at 25 °C with a thermostatically controlled cell holder attached to Neslab's RTE—110 water bath with an accuracy of ±0.1 °C. Spectra were collected with scan speed of 20 nm/min and response time of 1 s. Each spectrum was the average of four scans. Far UV CD spectra were taken at protein concentrations of 0.3 mg/ml with 1 mm length cell.

2.2.6. Dynamic light scattering (DLS) measurements

DLS measurements were carried out at 830 nm by using DynaPro-TC-04 dynamic light scattering equipment (Protein Solutions, Wyatt Technology, Santa Barbara, CA) equipped with a temperature-controlled micro sampler. SBA (10 μM) was incubated in different conditions for overnight. Subsequently, samples were spun at 10,000 rpm for 10 min and were filtered serially through 0.22 and 0.02 μm Whatman syringe filters directly into a 12 μl quartz cuvette. For each experiment, 20 measurements were taken. Mean hydrodynamic radius (R_h) and polydispersity were analyzed using Dynamics 6.10.0.10 software at optimized resolution. The R_h was estimated on the basis of an autocorrelation analysis of scattered light intensity data based on translation diffusion coefficient by Stoke's-Einstein relationship:

$$R_h = \frac{kT}{6\pi\eta D} \quad (2)$$

where R_h is the hydrodynamic radius, k is Boltzmann constant, T is temperature, η is the viscosity of water and D is diffusion coefficient [20].

3. Results and discussion

3.1. Acrylamide quenching measurements

Acrylamide quenching studies have been used to evaluate the exposure of tryptophan (Trp) residue in different states of protein [21]. The results were analyzed according to Stern–Volmer equation (Stern–Volmer plot represented in Fig. 1). Values of Stern–Volmer constant, K_{sv} are represented in Table 1. For complete exposure of Trp residues, K_{sv} value of 6 M GuHCl was used as reference (13.4 M^{-1}). Higher value of K_{sv} observed at pH 2.2 than that of at pH 7, reveals a more exposed Trp residue as compared to native state. Decrease in K_{sv} value from guanidine denatured SBA to

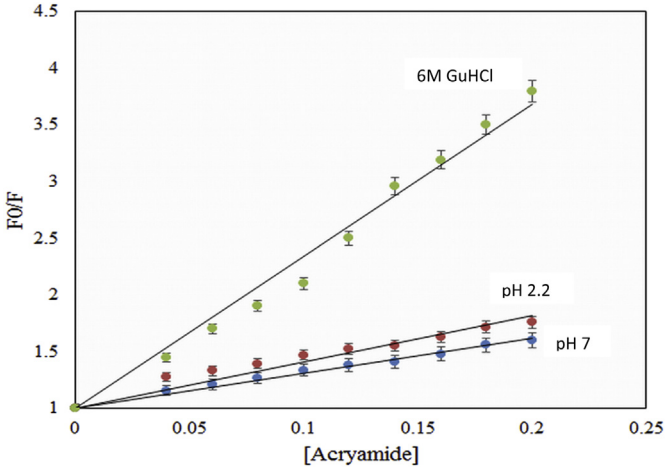

Fig. 1. Analysis of the acrylamide quenching data of SBA at different states (native at pH 7, molten globule at pH 2.2 and in presence of 6 M GuHCl) by Stern–Volmer plot.