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ORIGINAL ARTICLE

Equilibrium Study of Bovine Serum Albumin with Coomassie Brilliant Blue R-250 at Different Temperatures for an Improved Bradford Protein Quantification Assay

[1]Md. Rashadul Islam, [1]Sabrina Amita Sony, [1]Sintheya Islam, [2]H. M. Syeed Uddin, [3]Apurba Kumar Sarkar, [1,4]Mostafa Kamal Masud &[1*]H. M. Syfuddin

[1]Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh

[2]Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

[3]Department of Microbiology, MRS. K.S.K. College, Beed, Maharashtra

[4]Institute for Superconducting and Electronic Materials, Australian Institute for Innovative Materials (AIIM), University of Wollongong, Squires Way, Innovation Campus, North Wollongong, NSW 2519, Australia

*Correspondence: syfuddin-bmb@sust.edu

ABSTRACT

Protein quantification in an aqueous solution is highly desirable in biochemical research with a variety of applications, ranging from enzymatic studies to biomarker detection for disease diagnosis and prognosis. Bradford protein assay is one of the highly acceptable methods in this regard for its high sensitivity, but also suffers interference from assay conditions. In this study, we investigated the thermal property of molecular interaction of Bovine serum albumin (BSA) and Coomassie brilliant blue R-250 under different bio-favorable conditions. Among different thermal states, room temperature showed promising agreement with the previous thermal study of BSA and the equilibrium of the reaction retained after 30 minutes. This preliminary study will be the key for establishing a new method for protein quantification regarding detection, diagnosis, and prognosis of various diseases.

Keywords;*Bradford Protein Assay, Bovine Serum Albumin, Coomassie Brilliant Blue R-250.*

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INTRODUCTION

Proteins are highly abundant molecules and play important roles in all cellular functions with their own specific tasks such as structural support, activity in body movement or in defense against germs. Hence, protein characterization methods and technologies have been the major focus because of increasing number of protein drug development, biomarker discovery and their prior existence [1]. Blood protein biomarkers currently play a crucial role to detect Alzheimer [2, 3], cancer [4], liver disease [5], heart disease [6], renal dysfunction [7] and prediction of other diseases [8].Various disease diagnosis and prognosis are relying on serum protein identification and quantification [9, 10]. Protein assay systems, for example, Lowry protein assay, colorimetric method, biuret and Bradford protein assay have been established for quantification of protein. Among the all, Bradford assay is the most reliable method for its rapid sensitivity [11, 12] and convenience [13, 14].

The Bradford assay is a spectroscopic analytical procedure, used to determine protein concentration in a solution. Various analytical and qualitative approaches have been developed after modifying Bradford assay [15-17]. Recently, the conjugated interaction between Bradford reagent(BR) and Bovine serum albumin (BSA) have drawn an increasing attention since phosphorylated adducts of albumin might be

used as the biomarker for cancer detection [18, 19]. Having 76% sequence homology Bovine serum albumin (BSA) is regarded as the closest and reliable source that can be used instead of Human serum albumin (HSA) at spectroscopic purposes [20, 21].

BSA-BR interaction might be the fundamental aspect where thermodynamics had a great impact. Molecular interaction conditions such as ionic strength [22-24], pH [23, 25] were undertaken to establish the thermal stability of BSA. Bradford Dye, Coomassie brilliant blue R-250 is widely used in this regard to the evaluation of BSA-BR binding interaction. As per the study, BSA has no such interference on spectroscopic variation that proposes quantification of BSA itself, while with dye shift of spectra, reveal the interaction as well as the quantity of BSA [26, 27]. No previous study has been performed to distinguish BSA-BR stability regarding thermodynamic aspect.

This study focuses to establish the thermodynamic status of BSA-BR interaction as well as to determine reaction equilibria. To attain these objectives, we carried out detailed investigations, regarding the interaction using UV-vis absorption spectroscopy and pH records. The outcome of this current study might be a consideration for the determination of protein quantity and demonstrates which thermal condition is ideal and how long it is applicable to examine various proteins containing the solution.

MATERIALS AND METHODS

Preparation of Bovine Serum Albumin Sample

Bovine Albumin V (powder) was used. It is a product of LOBAL chemie, microbiological grade. For the preparation of BSA solution, 25 ml of distilled water was added to 250 mg of Bovine Serum Albumin sample. The sample was prepared at an optimized concentration of 10mg/ml.

Preparation of Bradford Reagent

A sample of Bradford reagent was prepared using 5 mg of Coomassie Brilliant Blue R-250 dissolved in the mixture of 2.5 ml 95% ethanol and 5 ml 85% phosphoric acid. The final solution was diluted with water to a final volume of 50 ml [28, 29].

Preparation of BSA Conjugated to Bradford Reagent

Three samples of different concentration of albumin coupled to different concentration of Bradford reagent 4:6, 5:5, 6:4 were prepared. To prepare 3ml of final volume for the BSA-BR 4:6 ratio, 1.2 ml of BSA solution was added with 1.8 ml of Bradford reagent. At the same way, the BSA volume of 1.5 ml, and 1.8 ml were used to prepare 5:5, 6:4 ratio solution respectively.

Measurement of UV-vis spectrum

The UV-visible absorption spectra were recorded at a UV-1800(Shimadzu Corporation) spectrophotometer equipped 1.5 cm plastic cell. The measurements were performed at different temperatures (room temperature- 25^0C, elevated- 34^0C - 44^0C and low temperature- 1^0C to 2^0C). Absorption spectra of BSA-BR were performed at wavelength 595nm [30].

pH Test

pH is an indicator to visualize chemical reaction. To perform this test PHS-25 (Linda Instrument) was used. All records were taken at different conditions (Temperature and concentrations).

RESULTS AND DISCUSSION

The Bradford assay, a colorimetric protein assay, is worked based on an absorbance shift of the dye [12]. Under acidic conditions the red-tinted form of the dye is converted into its bluer form by binding to the protein during the assay. In acidic conditions, the dye binds to proteins through basic amino acids (primarily arginine, lysine and histidine), and the number of Coomassie dye ligands bound to each protein molecule is approximately proportional to the number of positive charges found on the protein. Protein-dye binding causes the dye to change from reddish-brown to bright blue [31].

For some potential ratio of the BSA-BR solution, 4:6, 5:5, 6:4; absorbance variation over time at different temperature were seen. At low temperature, nearly 0^oC, absorbance fluctuates rapidly where high temperate (34^oC – 44^oC) showed transition state for the first 10 minutes. But at room temperature, it followed a slowly changing state. This indicates that BSA in room temperature have higher affinity to react with BR where increasing temperature shows some transitional period at the initial phase. At very low-temperature absorption spectra of BSA-BR faces uneven fluctuation that elucidates improper interaction of the protein with dye.

Analyzing those figures 1 (a, b and c), three ratios of BSA-BR could react in three deferent conditions resulted with different UV-vis absorption spectra. At room temperature, Bovine Serum Albumin binds with Bradford reagent and simultaneous binding faces homogeneity throughout the reaction period. BSA-

BR interaction at elevated temperature has the same manner as room temperature. It shows sharp rising at the initial stage to synchronize with elevated temperature and gradually it attains homogeneity. The interaction shows heterogeneity and the bindings are not spontaneous at low temperature.

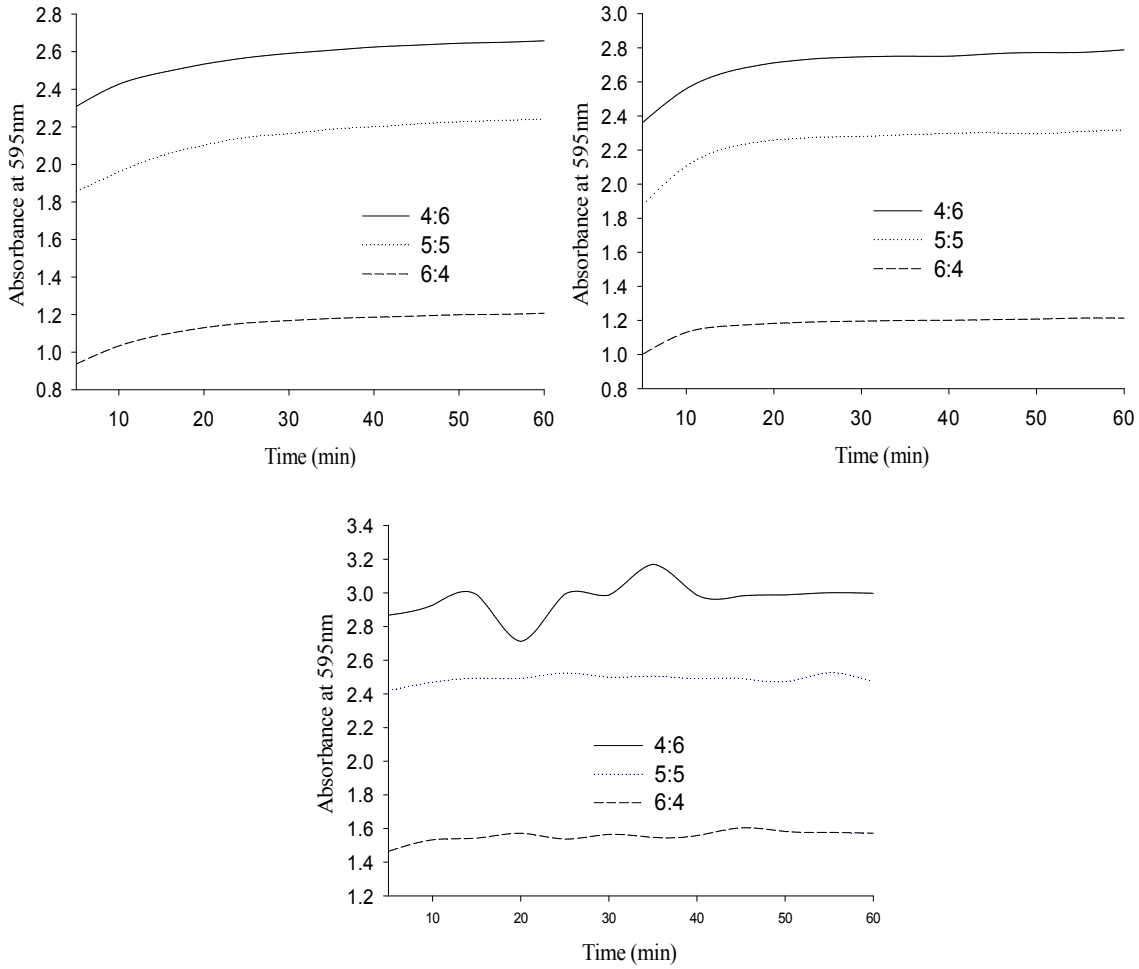

Figure-1 Absorbance of BSA-BR composition with different ratios are plotted against time at (a)room temperature (25^0 C), (b) elevated temperature (34^0C-44^0C),(c)low temperature (1^0C-2^0C).

BSA shows a significant changing status while reacting with Bradford reagent. It reacts simultaneously with BR for first 30 minutes. After 30 minutes, it faces equilibria and the UV-vis absorption spectrum shows dynamic consistency. All absorption spectra and pH records demonstrate the indication.

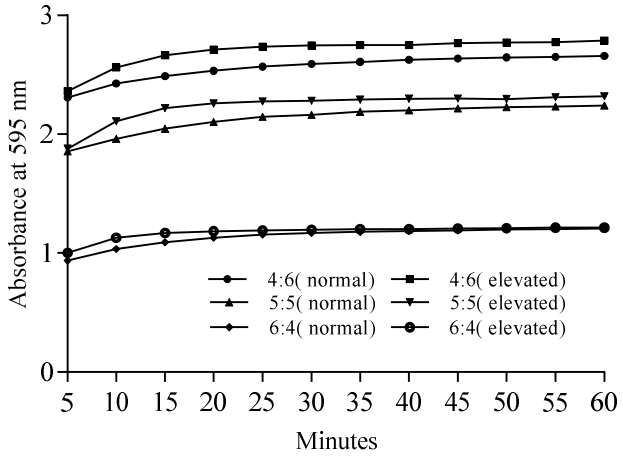

Figure-2: UV-vis absorption spectra of different ratios of BSA- BR against time.

UV-vis absorption of 4:6, 5:5 and 6:4 of BSA-BR resulted with an influential guideline. In figure-2 all the spectra of absorption against time face dynamic equilibria after a certain time and the time is 30 min. At the initial stage, BSA starts to react and the absorption increases with time. But after 30 min, BSA got saturated by BR and for that reasons, their interaction produces a horizontal curve for the rest of observation.

pH records of these three different ratios show that in all cases, the reaction rate increases with time until achieving equilibria, denoting decreasing curve (shown in Figure-3). Afterward, the reaction rate shows consistency with time. This indicates that after conjugation, BSA starts to bind with BR swiftly but when BSA got saturation by BR, the rate slows down. As the reaction is not over at equilibrium state, the BSA-BR simultaneously react and result with consistent output [32].

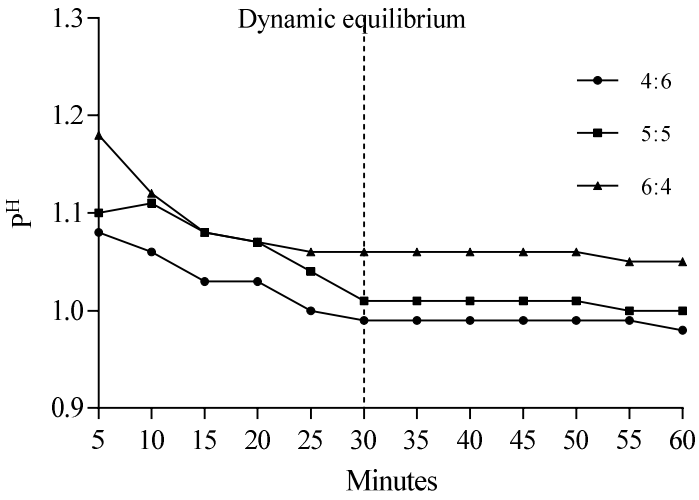

Figure-3: pH is plotted against for various ratios of BSA-BR at normal temperature.

This present study recommends that the room temperature, 25^0C is satisfactory for investigating the proper interaction of BSA-BR while their dynamic equilibria reach after 30 minutes. These findings will be the prerequisite for establishing various protein detection and characterization, especially in protein-based biomarker design.

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