Lyallpur Khalsa College
Jalandhar-144001, Punjab

Research & Reviews in Biotechnology & Biosciences
Homepage: http://www.biotechjournal.in
Volume: 6, Issue: 1, Year: 2019 PP: 1-4

Notes & Tips

Fluorescence spectroscopy based folding-unfolding of Protein [Bovine Serum Albumin (BSA)]: an UG/PG experiment in Biotechnology

Arun Dev Sharma[3], Priya Nischal, Narvir Singh[1], Jagpreet Singh Nanda[2]*

[3]PG Dept of Biotechnology, Lyallpur Khalsa College, Jalandhar, Punjab, India

[1]PG Dept of Physcis, Lyallpur Khalsa College, Jalandhar, Punjab, India

[2]Staff scientist, LMRPS, NICHD, National Institutes of Health, RM 2A03 bldg 49, 9000 Rockville pike, Bethesda MD, USA

https://doi.org/10.6084/m9.figshare.8988713.v2

Article History

Received: 02/01/2019
Revised: 25/01/2019
Accepted: 15/02/2019

**Corresponding Author:*
E-Mail:
arundevsharma47@gmail.com

Abstract

Eukaryotic and prokaryotic cells contain number of endoflores like proteins which undergoes folding-unfolding process and play very crucial biological role inside the cells. In this study an under-graduate (UG) and post-graduate (PG) experiment was set up to study folding-unfolding of protein BSA. It was observed that fluorescence of the protein in solutions varied as per the concentration of urea and guanidine hydrochloride. It was postulated that fluorescence spectroscopy based approach can be used to document protein folding process to the students of UG and PG classes.

Introduction

The study of protein structure and properties is a key part of all the basic courses in Biotechnology. Among all, one of the most important concepts in the area of protein biochemistry is the study of native and denatured states of the macromolecules through folding-unfolding process. It was reported that each protein exists as an unfolded state or random coil when translated from a sequence of mRNA [1]. This polypeptide lacks any stable (long-lasting) three-dimensional structure. Upon synthesis from ribosome, a linear chain begins to fold into its three-dimensional structure. The correct three-dimensional structure is essential to function, although some parts of functional proteins may remain unfolded, so that protein dynamics is important [2]. Failure to fold into native structure generally produces inactive proteins, but in some instances mis-folded proteins have modified or toxic functionality.

Eukaryotic and prokaryotic cells contain a number of compounds that are fluorescent when excited with UV light. Most important endogenous flurophores are molecules widely distributed in cells and tissues, like proteins containing aromatic amino acids. Many enzymatic cofactors, such as FMN, FAD, NAD and porphyrins, which are also intrinsically fluorescent, add to the protein fluorescence play important roles in the cellular energy metabolism. These moieties have a common trait in that they all contain

aromatic ring structures that absorb UV light for excitation. The experiment demonstrated here have been developed as a part of overall revision of advanced courses in biochemistry and biotechnology which highlight the importance of physical and analytical chemistry to study the structures and properties of biological macromolecules. The experiments reported here involve the use of fluorescence spectroscopy to determine the folding unfolding of protein BSA by denaturants like urea and guanidine hydrochloride.

Material and methods

Stock solutions

Approximately 10 M Urea (CDH) was prepared in 0.05 M citrate buffer, pH 3.0. The flourescence background of this solution was measured. Stock solutions of 8 M guanidine hydrochloride (SRL) were made in 0.1 M tris buffer, pH 7.0 and used without further purification. The final concentrations of denaturants were 2 and 4 M.

BSA solution

About 0.12 mM solution of bovine serum albumin (BSA) was prepared in citrate and tris buffer, as appropriate.

Fluorescence spectroscopy analysis

A series of solutions containing a fixed amount of BSA (3 μM) and varying concentration of urea and guanidine hydrochloride (2-4 M) were prepared from stock solutions. The final volume of each sample was 1.0 ml. the solutions were allowed to sit at least for 1h and the fluorescence spectrum of each sample was then measured on Perkin Elmer Spectrophotometer (FL6500). The excitation wavelength was 280 nm and fluorescence emission spectrum was recorded over wavelength range from 300-400 nm. All experiments were done at room temperature (~30^{o}C).

Results and Discussion

Proteins are fundamental building blocks inside the Eukaryotic and Prokaryotic cells. Correct folding of proteins during the process of translation play a very apt role in various biological processes. It was always assumed that inside the cells, proteins like always exits only in two states, the native (N) and completely unfolded or denatured (D) [3]. These two states always exit in equilibrium. Like most other flurophores, proteins when excited with a suitable UV wavelength, produce fluorescence. Therefore, in order to study folding-unfolding process, bovine serum albumin (**BSA**) was chosen. Typical fluorescence spectra for native and unfolded BSA in urea and guanidine hydrochloride is shown in Fig 1A/B.

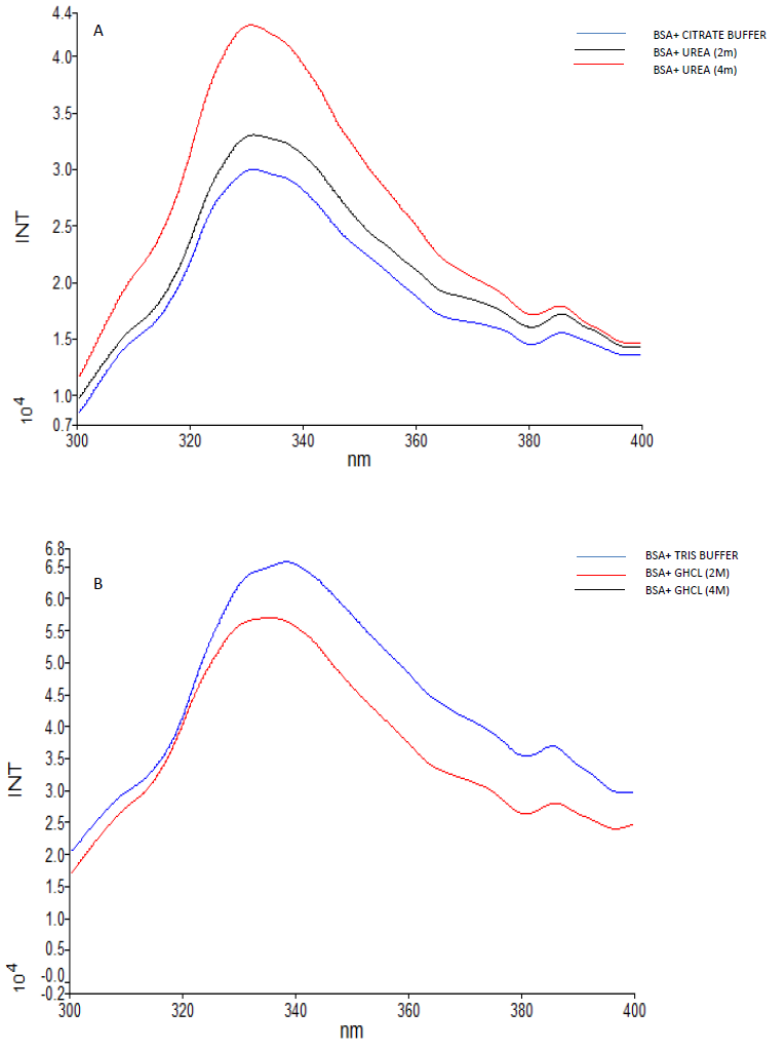

Fig 1: Fluorescence emission spectra of BSA with denaturants. Excitation λ: 280 nm

It was observed that as the concentration of denaturant increased (both urea and ghcl), the fluorescent yield also increased dramatically. BSA, like most globular proteins, contains aromatic amino acids. The three amino acid residues that are primarily responsible for the inherent fluorescence of proteins are tryptophan, tyrosine and phenylalanine. These amino acids are known to be fluorescent, but tryptophan (Trp-134, Trp-213, Trp-214) fluorescence dominates usually the fluorescence of the macromolecules. Furthermore when, when BSA is denatured, the exposure of tryptophan residues in the hydrophobic core of the molecules results in the fluorescence increase [4]. Hence the fluorescence intensity may be then be used as a measure of the extent of unfolding of the protein.

To conclude, this experiment provides opportunity to UG and PG students of life sciences to monitor folding- unfolding process and reinforcement of important

concepts regarding protein folding. The students can determine experimentally that folding unfolding in thermodynamically favorable process and in lab report they should be asked to discuss various factors like solvent, temperature etc that contribute to folding unfolding process.

Acknowledgment

ADS want to thank LKC management for this support. In addition, ADS want to thank DST, Govt of India for sanctioning the fluorescent spectrophotometer under FIST grant.

Conflict of interest: no

REFERENCES

1. Jagannathan B, Marqusee S. 2013. Protein folding and unfolding under force". Biopolymers. 99: 860–9
2. Hartl FU, Bracher A, Hayer-Hartl M. 2011. Molecular chaperones in protein folding and proteostasis. Nature. 475: 324–32.
3. Basak S., Chattopadhyay K. 2014. Studies of protein folding and dynamics using single molecule fluorescence spectroscopy. Phys. Chem. Chem. Phys. 16:11139–11149
4. Royer C.A. 2006. Probing protein folding and conformational transitions with fluorescence. Chem. Rev. 106:1769–1784