Equilibrium unfolding of bovine serum albumin monitored using tryptophan fluorescence

Theory / Principle:

The folded structure of a protein is crucial for its function. The factors that govern protein folding are manifold. They include the protein sequence and other external parameters like solvent polarity, temperature, pH and salt concentration. It is still not possible to unambiguously predict the 3 dimensional structure of a protein from its primary sequence.
In this laboratory we monitor the equilibrium unfolding of bovine serum albumin by monitoring the fluorescence intensity of the lone Trp in the protein. The Trp fluorescence intensity and emission wavelength serve as probes for the environment surrounding the Trp residue in the protein.

Methodology :

(a) Requirements:

(i) Native buffer : 100 mM NaH_2PO_4, pH 7

(ii) Unfolding buffer: Three different concentrations of Urea, 2 M, 4 M and 6 M, is prepared in 100 mM Sodium phosphate buffer (NaH_2PO_4), pH -7.0

(iii) Protein stock solution : Bovine serum albumin (BSA) at 0.5 mg/ml is prepared in 100 mM Sodium phosphate buffer, pH 7.

Glasswares / Plasticwares:

- Eppendorf tubes (1.5 ml) - 10 Nos.
- Micropipettes (P1000, P200)
- Microtips (1000 µl, 200 µl)
- Quartz cuvette

Equipments:

- Vortex Mixer
- Spectrofluorimeter

Procedure:

1. Aliquot buffer and protein in eppendorf tubes as indicated in the table below.

Sl No.	Sample Name	Buffer		BSA
		Name	Quantity (ml)	Quantity from BSA stock (0.5 mg/ml) (ml)
1.	Blank 1	NaH_2PO_4 buffer	1.0	…………….
2.	Sample 1	NaH_2PO_4 buffer	0.8	0.20
3.	Blank 2	2 M Urea buffer	1.0	……………..
4.	Sample 2	2 M Urea buffer	0.8	0.20
5.	Blank 3	4 M Ureabuffer	1.0	…………….
6.	Sample 3	4 M Urea buffer	0.8	0.20
7.	Blank 4	6 M Urea buffer	1.0	…………….
8.	Sample 4	6 M Urea buffer	0.8	0.20

2. Seal with parafilm and vortex vigorously.
3. Incubate all samples for 12-20 hours at room temperature.

Setting up the Spectrofluorimeter:

Experiment shall be performed at 25° C on a spectrofluorimeter. Set the excitation wavelength at 295 nm and the emission wavelength range from 315 to 415 nm. Set the excitation bandwidth at 1 nm and the emission bandwidth at 3 nm.

Observations:

1. Measure fluorescence emission spectrum of Blank1.
2. Under the same conditions measure the emission spectrum of sample1 (0 M Urea).
3. Repeat collection of sample spectra one more time.
4. Store results.
5. Repeat steps 1 to 4 with blanks and samples for 2 M, 4 M and 6 M Urea concentrations.

Calculations:

1. Subtract the blank 1 spectrum from sample 1 spectrum to correct for Raman Scatter. Repeat this vice versa for sample 2, sample 3 and sample 4 spectra also.
2. Measure area under the spectrum and plot your values in a tabular form as under-

[Urea]	Area	
0 M	1.	2.
2 M	1.	2.
4 M	1.	2.
6 M	1.	2.

3. Plot a graph between [Urea] vs Area
4. Measure the wavelength with maximum emission as a function of Urea concentration and plot your values in a tabular form as under. Also plot the graph between [Urea] vs λ_{max} emission spectra.

[Urea]	λ_{max} emission
0 M	
2 M	
4 M	
6 M	