Poster Presentation
Inter-Disciplinary Explorations in Chemistry (I-DEC 2018)

Spectroscopic Probing of the Refolding of an Unfolded Protein Through the Formation of Mixed-Micelles

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Abstract: Investigating the refolding of proteins from their denatured states to their thermodynamically stable native states under *in vitro* conditions has always been a great challenge.[1] Here, we report the unfolding of the globular protein, Bovine Serum Albumin (BSA) induced by anionic surfactant sodium dodecyl sulphate[2-3] (SDS) and subsequently monitored the refolding of this denatured BSA using triblock copolymers F127 and P123 through the formation of mixed micelles.
Our study exclusively represents the reversibility of this unfolding-refolding process using pluronic triblock copolymers F127/P123 as refolding agents. We confirm the recovery of its native state from its denatured state estimating the α-helical structure of the denatured protein from the CD data which nicely support our steady state fluorescence spectra monitoring the fluorescence of the intrinsic Trp molecules present in BSA. We have also carried out time resolved study to get an idea about the excited state lifetime and this data also corroborate the stepwise recovery of the denatured BSA as well as the reversibility of the processes. ITC data explain the negligible interactions between the triblock copolymers and the native state of BSA. The high binding constant of SDS and triblock copolymers probably play the crucial role in the stepwise recovery of the unfolded BSA followed by reversibility of the unfolding-refolding processes through the formation of the mixed micelles. The mechanism of mixed-micelle formation has been substantiated by the fact that the Guanidine Hydrochloride denatured BSA does not react with F127/P123 and hence no recovery of the protein was observed.

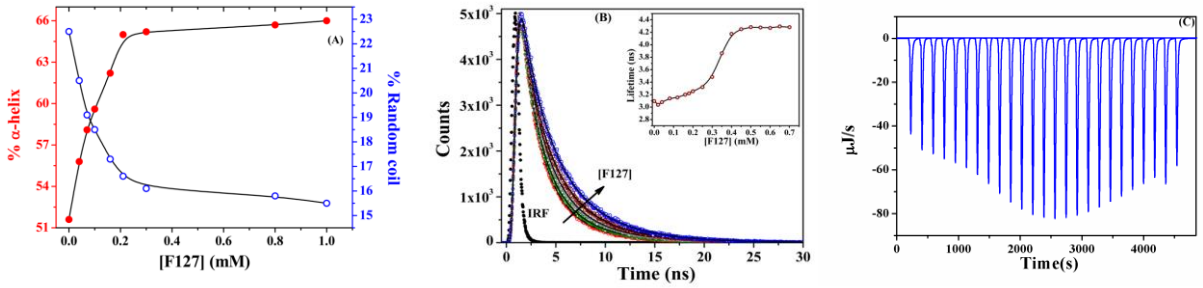

Figure: (A) Plot of variation of the α-helix percentage (-○-) and random coil (-○-) of unfolded BSA with increasing concentrations of F127. (B) Fluorescence lifetime decay spectra of SDS induced denatured BSA with various concentrations of F127. Inset shows the variation of the average lifetime of unfolded BSA with increasing concentrations of F127. (C) Integrated heat change data from ITC for SDS induced denatured BSA with F127 at 25 °C (pH 7.4) after the subtraction of heat of dilution.

References:

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