Effect of Crowding Environment on Protein Kinetics and Dynamics using Fluorescence as the basic technique

Laxmipriya Prusty, Suchismita Subadini and Harekrushan Sahoo*

Biophysical and Protein Chemistry Lab, Department of Chemistry, National Institute of Technology, Rourkela-769008

Email*: sahooh@nitrkl.ac.in

Abstract

Protein folding, the issue of protein stability and dynamic perturbations, is one of the fundamental importance in the living cell and the biological activities. While the polypeptide chain folds with the help of chaperone in the ribosome, the cytoplasmic component (which includes both micro-and macromolecules) influences the protein folding pathway and their dynamics. To understand their effect on the folding and dynamics, it is very much essential to monitor the effect of each component on the protein local as well as global structure and conformation. Out of the cytoplasmic components (i.e., micro and macro-molecules), we have investigated the effect of small molecules on the protein dynamics process. These small molecules in the form of inorganic salts (i.e., cations/anions: concentration of ≈150 mM) constitute 0.3 % of the total volume of the cell. For this purpose, cellular retinoic acid binding protein I (CRABP I) is used as a model protein. FCS observations revealed the impact of anions on the diffusion of protein as well as the hydrodynamic radius at room temperature. The outcome of an FCS experiment is a correlation function between intensity and time of diffusion of CRABP I in the presence of different salts. Taking a note from the impact of micromolecules, we have started observing the effects exerted by the macromolecules, such as carbohydrates (i.e., glucose, sucrose, fructose or the synthetic ones like Dextran, Ficoll and Cyclodextrin), which has an approximate concentration of ≈ 300 to 400 mg/ml in the cytosol of Escherichia coli and constitutes 20 to 40 % of the total volume, on the nature of the folding intermediates. Starting with our objective, we are trying to see how the shape and size of macromolecule bring changes in the kinetics.

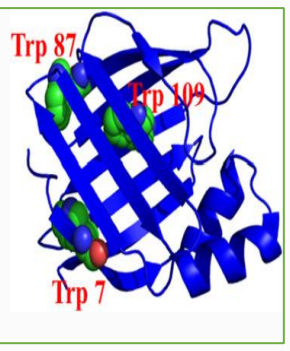
Introduction

Cellular retinoic acid binding protein I

PDB ID: 1CBR

- 136 amino acids
- Three intrinsic Trp
- Predominantly aβ-sheet protein
- Three cysteines (Cys 81, Cys 95 and Cys 129)

Transports RA from cytoplasm to nucleus

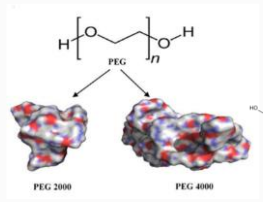


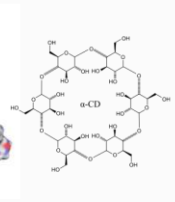
Micro molecules:

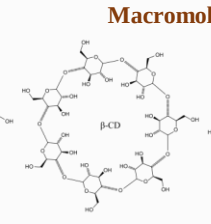
Different inorganic salts

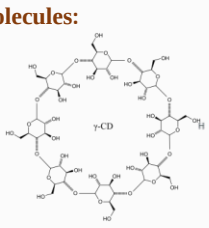
Na_2HPO_4, NaCl, Na_2SO_4

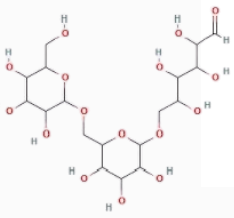
Macromolecules:

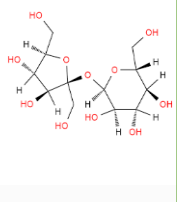








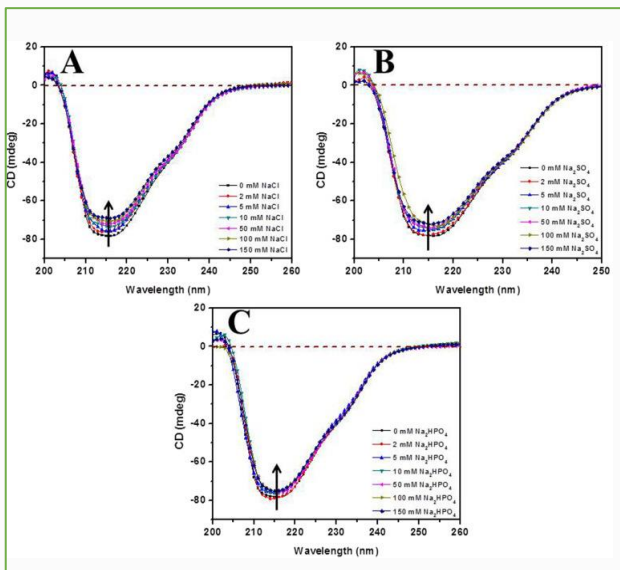




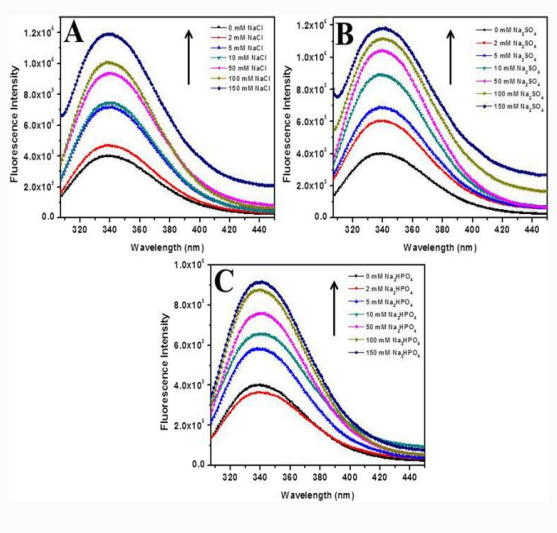
PEG(Poly ethylene glycol) CD (Cyclodextrin) Dextran Ficoll

Results and Discussion

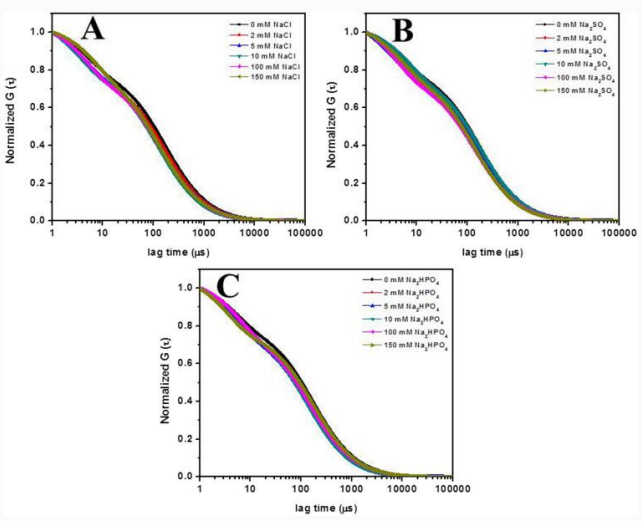
Effect of micro-environment



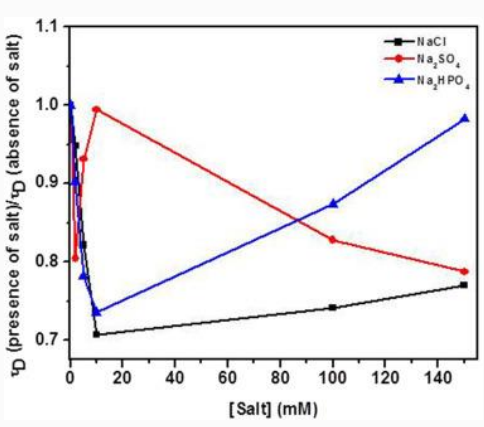
Secondary structure analyses of CRABP I ;CD spectra changes



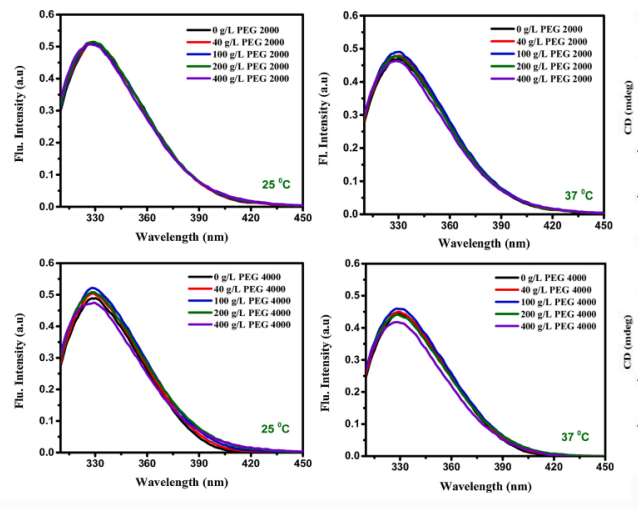
Emission spectra of CRABP I; fluorescence intensity increases



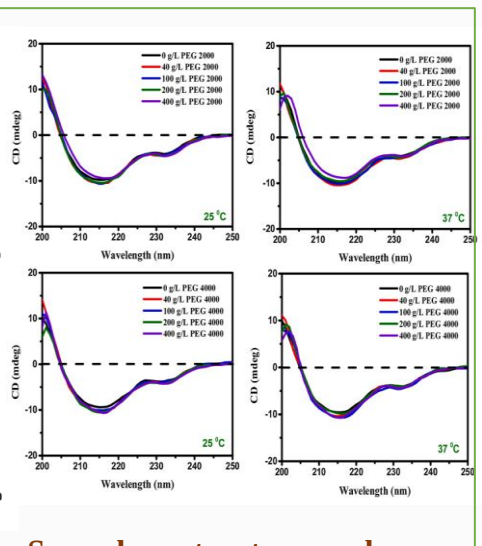
FCS analysis of CRABP 1; diffusion time changes



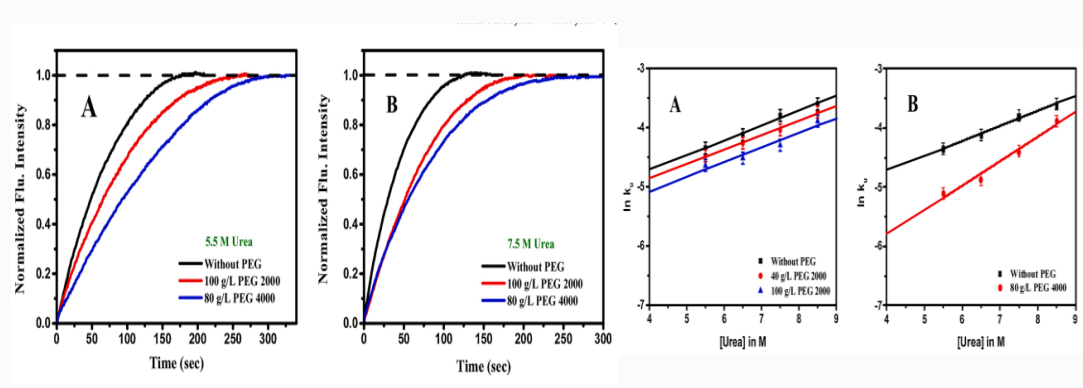
Effect of macro-environment



Emission spectra of CRABP I in PEG 2000, 4000; Local environment of Trp



Secondary structure analyses of CRABP I; CD Spectra are remaining intact



Unfolding kinetics of CRABP I at various concentrations of PEG 2000; Retardation of protein unfolding rate

Conclusion

- **Anions of micromolecular crowder bring higher alteration in the conformation of CRABP I.**
- **From stability and kinetics using fluorescence, PEG act as a stabilizer in unfolding kinetics of CRABP I.**

References

- Colloids and Surfaces B: Biointerfaces, 2021, 202, 111696, 10.1016/j.colsurfb.2021.111696 .
- Journal of Molecular Liquids, 2020, 320, 114489, 10.110.1016/j.molliq.2020.114489 .

Acknowledgement

DAAD Deutscher Akademischer Austauschdienst German Academic Exchange Service

SERB DIA

inspire विज्ञान एवं प्रौद्योगिकी विभाग DEPARTMENT OF SCIENCE & TECHNOLOGY