Impact of Glycosaminoglycans (GAGs) on Stability and Kinetics of Bone Morphogenetic Protein-2 (BMP-2): A Biophysical Approach

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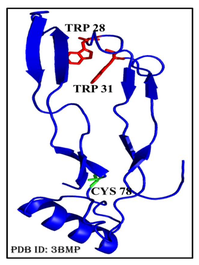
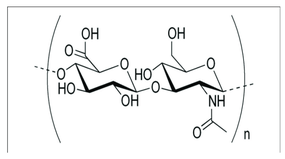
ABSTRACT

Bone morphogenetic protein-2 was overexpressed, purified and refolded using gradient dialysis. The stability and kinetics of the BMP-2(bone morphogenetic protein-2) was studied in presence and absence of glycosaminoglycan (Hyaluronic acid and chondroitin sulphate).The experiments are carried out using UV-Vis spectrophotometer, spectrofluorimeter in both wavelength scan and time scan modes, circular dichroism. The kinetics mode was used at 37°C to mimic the cellular environment. Absorbance data and fluorescence intensity confirmed the quenching in both CS (chondroitin sulphate) 1 and HA (hyaluronic acid). The secondary structure of the BMP-2 was significantly changed upon interaction with both HA and CS which introduced more compactness, confirmed by circular dichroism data. Unfolding kinetics of BMP-2 in presence of CS confirmed that the rate of association decreased in presence of 15μM urea. Further the unfolding kinetics of BMP-2 in presence hyaluronic acid and sulphated hyaluronic acid using urea (6M and 8M) suggested an opposite trend. In presence of sulphated HA the rate of unfolding decreased on increasing concentration of HA (0.1 mg/ml, 0.25 mg/ml, 0.5 mg/ml.) and the opposite trend was observed in HA on increasing the concentration (0.1 mg/ml, 0.25 mg/ml, 0.5 mg/ml.). This study indicates the role of different glycans in proper functioning of BMP-2 can be helpful for further in vivo studies.

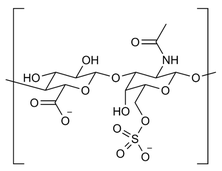
INTRODUCTION

- A growth factor of the transforming beta family (TGF-β).
- It consists of 2 polypeptides, each of 114 amino acids.
- Secondary structures: 42% β-sheet & 10% α-helices.
- 2 tryptophan residues (monomer).
- Cysteine 78 contributes to the formation of biologically active dimer BMP-2.
- Have osteoinductive properties





Hyaluronic acid

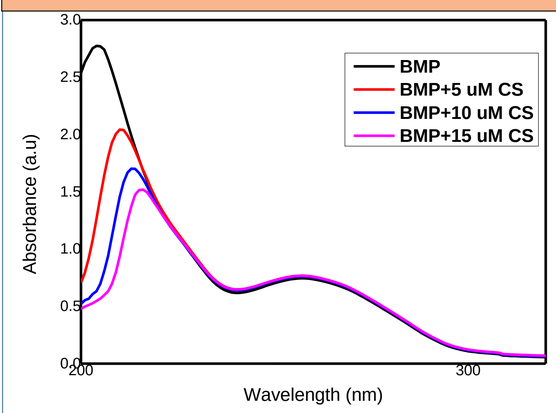


Chondroitin sulfate

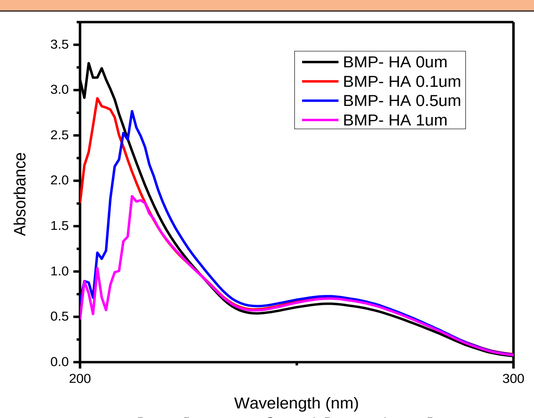
- Glycosaminoglycans (GAGs) or mucopolysaccharides are long, linear polysaccharide consisting of repeating disaccharide units
- GAGs help in cell hydration and structural scaffolding, cell signaling, which serves to modulate a vast amount of biochemical processes.eg. Chondroitin sulfate, hyaluronic acid etc.
- These are found in bone ECM and helps proteins like BMP-2 and others in proper functioning and provide scaffolds.

RESULTS AND DISCUSSION

ABSORPTION SPECTRA

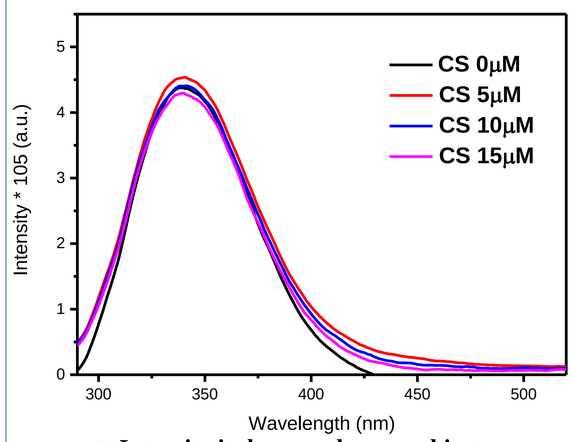


- Absorbance of amide region decreases
- CS is polar – having a strong affinity for water
- Lowers the energy – shift towards higher wavelength

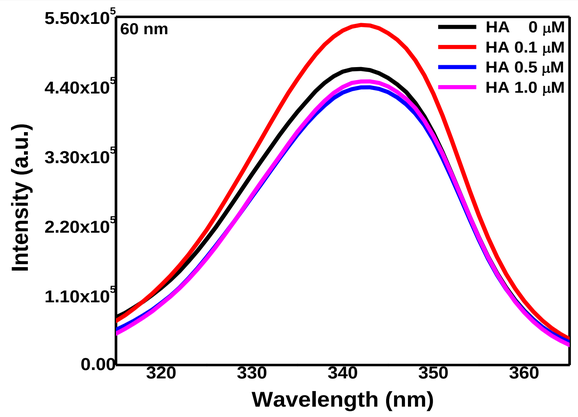


- Absorbance of amide region decreases.
- A shift is observed on increasing HA

EMISSION SPECTRA

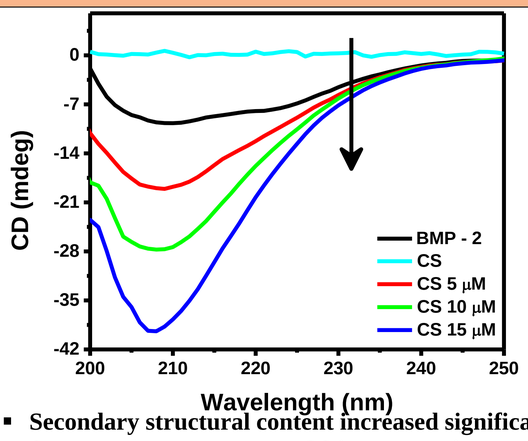


- Intensity is decreased – quenching
- No shifting

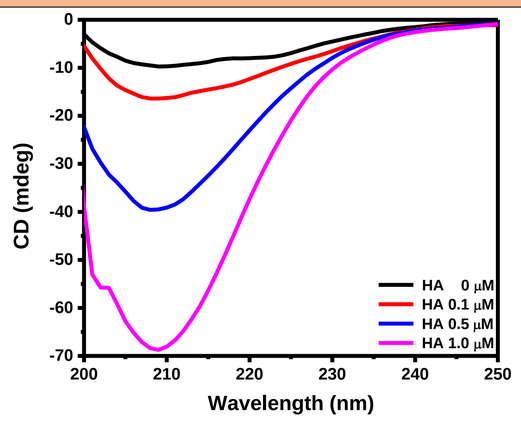


- Intensity is decreased – quenching
- No shifting

CIRCULAR DICHROISM SPECTRA

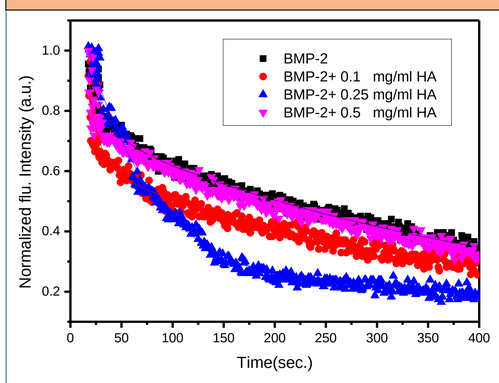


- Secondary structural content increased significantly
- Structural compactness – CS interacts with water
- ~3nm blue shift of spectra – viscosity of CS

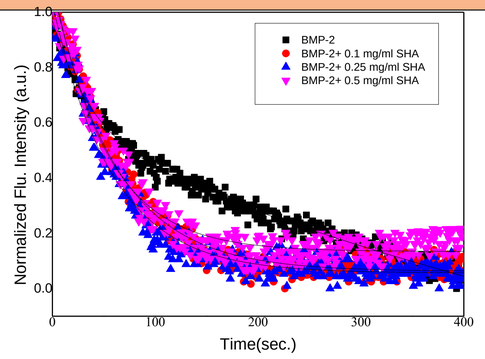


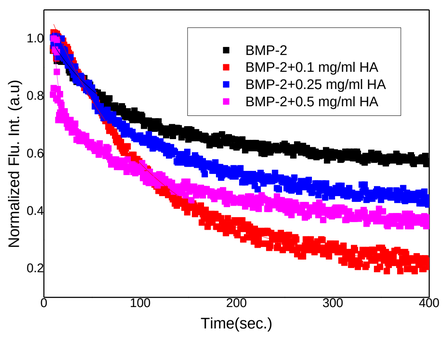
- Secondary structural content increased significantly

UNFOLDING KINETICS

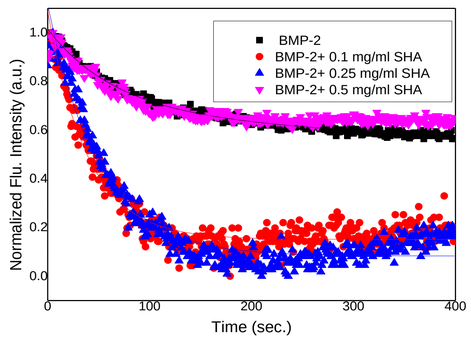


6M





8M



- Rate of unfolding decreases on increasing concentration of sulfated hyaluronic acid and opposite trend was observed in case of Hyaluronic acid. Rate of unfolding was faster in 8M urea concentration.

CONCLUSIONS

- UV spectra shows interaction as absorbance at amide region decreases.
- Quenching was seen from the fluorescence spectra.
- Secondary content of the protein increases upon interaction.
- Sulfated hyaluronic acid accelerated the unfolding process while opposite case was seen in the Hyaluronic acid.

REFERENCES

1. Rane. A.M., *Protein Expression and Purification* 90 (2013) 135–140.
2. Konar, M.; Sahoo, H.*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2020*, 242,118726
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