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Topic of Research	Effect of Macromolecular Crowding Agents and their Monomers on the Aggregation Pattern and Thermodynamics of Proteins

In this study, we sought to unravel the fundamental mechanisms underlying protein aggregation within two globular proteins namely, ribonuclease A (RNAase-A) and alpha lactalbumin (α-LA). Recognizing the intricate nature of the cellular environment, we employed macromolecular crowding agents to mimic the crowded conditions within cells. The rationale behind this approach was to observe how these agents influence the patterns of protein aggregation, providing valuable insights into the impact of the crowded intracellular milieu on this critical biological process. Moreover, contemporary research has highlighted the protein-stabilizing properties of osmolytes. Intriguingly, we aimed to investigate whether these osmolytes could exert inhibitory effects on the aggregation of proteins. The rationale behind exploring osmolytes lies in their potential as stabilizing factors, and understanding their impact on protein aggregation could pave the way for novel interventions.

Research Findings:

1. *Interaction of Extrinsic Fluorescent Dyes with Additives:*

- ThT and ANS dyes show strong binding affinities with macromolecular crowding agents (D-70 and F-70) and sugar osmolytes (glucose and sucrose).
- From molecular docking it was found that macromolecular crowding agents, especially Ficoll- 70 showed higher binding affinity.
- Fluorescence measurements confirm these interactions.
- TEM imaging reveals globular structures resembling protein aggregates.

Therefore, it is this study provides the importance of considering dye-additive interactions to avoid false positives while studying protein behavior studies.

2. *Impact of Macromolecular Crowding Agent and Glucose on Protein Aggregation:*

- D-70 enhances RNase-A aggregation.

- Glucose inhibits RNase-A aggregation and stabilizes the protein in its native conformation.
- Dual inhibitory action of glucose against thermal and D-70-induced aggregation.
- Glucose mitigates RNase-A fibril-induced cytotoxicity.
- Inconclusive results with α-LA led to focus on glucose, which prevents and slows thermally induced aggregation.
- Glucose inhibits toxic amyloidogenic cores and amorphous aggregates in α-LA.

3. *Structural Dynamics of Proteins under Thermal Aggregation:*

- D-70 promotes disordered aggregate formation in RNase-A.
- Glucose reverses disordered aggregates into ordered monomeric native forms, thus providing stabilization to the proteins.
- Far UV-circular dichroism (CD), Fourier-transform infrared (FTIR), and UV-vis spectroscopies were utilized to analyze secondary structure changes.
- The study suggests glucose's potential as a therapeutic agent for protein aggregation-related disorders, presenting a novel approach to drug development.

4. Protein Aggregation Dynamics and Anti-Aggregatory Effect of Glucose

- Temperature-induced aggregation of RNase-A and α-LA was studied by using advanced imaging techniques (AFM and TEM).
- Molecular docking reveals D-70 induces RNase-A aggregation and disrupts enzyme function.
- Glucose shows anti-aggregatory effects and potential as a potent inhibitor of protein aggregation.
- Comprehensive approach provides insights into protein stability and therapeutic interventions for aggregation-related disorders.

By delving into the inhibitory effects of these compounds on protein aggregation, our study not only contributes to the basic understanding of the molecular events involved but also lays the groundwork for potential therapeutic interventions in diseases associated with abnormal protein aggregation. This research has the potential to uncover strategies for mitigating the detrimental effects of protein misfolding and aggregation, particularly in the context of diseases where these processes play a central role.