Investigating the Conformational Ensembles of Intrinsically Disordered IA3s Proteins

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Presented: July 26, 2024, SSTP University of Florida

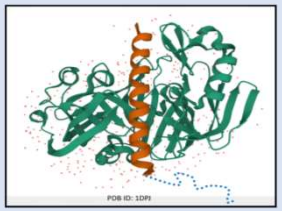
Abstract

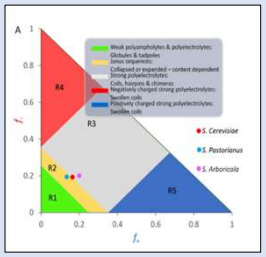
This work **examines the structural properties** of **IA_3** ***Saccharomyces pastorianus*** and ***Saccharomyces arboricola*** proteins with **Circular Dichroism** (CD) spectroscopy to establish a **correlation between their intrinsic parameters** and the **CD-identified structure**. CD spectra were obtained across varying concentrations of 2,2,2-Trifluoroethanol (TFE), revealing distinct structurally linked spectra features. **IA_3** ***S. pastorianus*** was observed to exhibit **consistent α-helical structures** across all tested TFE volumes, whereas **IA_3** ***S. arboricola*** exhibited **primarily α-helical structures but** had **minor deviations at lower TFE** concentrations. The **BeStSel** Single Spectrum Analysis tool was used to **find** the **quantitative structural compositions** from the CD data to highlight spectral characteristics representative of α-helical content. This investigation **provides notable insights** into the **structural composition** of **IA_3** ***S. pastorianus*** **and** ***S. arboricola*** and it establishes a foundation for **subsequent investigations** into their functional and structural mechanisms, as well as wider implications for proteins with inherent disorder.

Introduction

Intrinsically disordered proteins (IDPs) have been linked with many human diseases such as **Alzheimer's** (AD), **Parkinson's** (PD), **dementia**, **Huntington's**, **amyloidosis**, and some **cancers**[1]. **IA_3** is a **68 amino acid IDP** naturally found in the yeast Saccharomyces genus. IA_3 takes **no structure** in its unbound standard physiological environment.

IA_3 is a YPrA **proteinase inhibitor** via binding an induced α-helix in the NTD while keeping an unsolved CTD. 2,2,2-Triflouroethanol (**TFE**) **induced the same structure** in IA_3 that it takes when binding to YPrA[2,3].

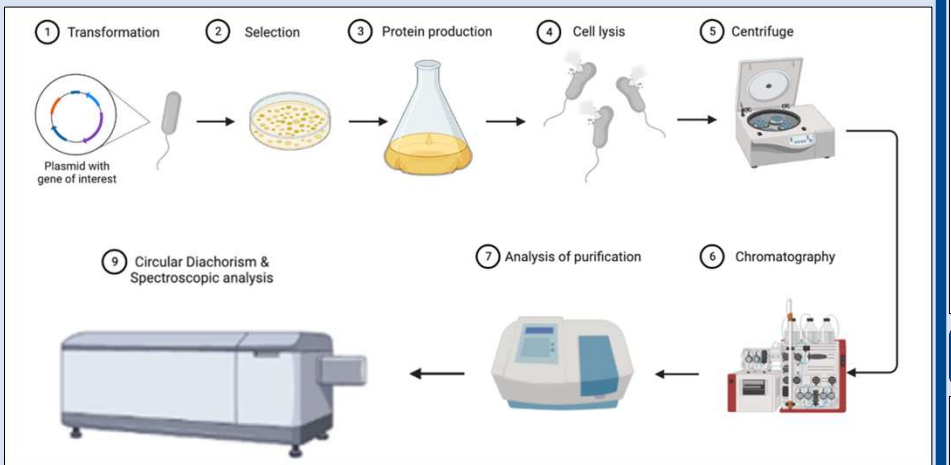




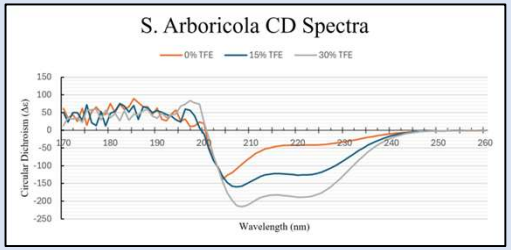
Circular Dichroism (CD) spectroscopy can **identify the secondary structural elements** (conformational ensembles) of the TFE activated IA_3 *S. pastorianus* and *S. arboricola* via the **BeStSel** Single Spectrum Analysis tool developed by József Kardos and András Micsonai.

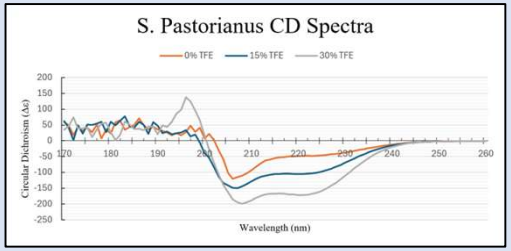
The **charge patterning** (κ), fraction of charged residues (**FCR**), net charge per residue (**NCPR**), and **Das-Pappu phase** are the intrinsic parameters calculated based on the sequence's physical features like charge and hydrophobicity with the **localCIDER** tool developed by the Pappu Lab. In this study we search for a **correlation between these intrinsic parameters and the structures** of *S. pastorianus* and *S. arboricola.*

Methodology



Results & Discussion





IA_3 proteins from S. pastorianus and S. arboricola were analyzed using BeStSel-analyzed CD data.

- ***S. pastorianus*** **IA_3** proteins showed α-helices across all TFE volumes.
- **S. arboricola IA3** proteins had 93.5% α-helix and 6.5% other structures at 0% TFE, and entirely α-helix at 15% and 30% TFE.
- The **unexpected result** for S. arboricola at 0% TFE suggests a potential need for further studies to confirm the structure.

We **were not able to identify the correlation or lack thereof** between the entirely α-helical structure observed in the TFE activated IA3 S. pastorianus or S. arboricola and the charge patterning, FCR, NCPR, or the Das-Pappu phase. Without further investigations into the positions of these structures across the protein and how the structures are arranged in 3d space, it is difficult to make any conclusions about how the charge patterning, the FCR, the NCPR, and the Das-Pappu phase impact that structure.

Conclusion

We've observed that the **methods** adapted **from** **IA_3** ***S. cerevisiae*** transformation, overexpression, and purification successfully **extend to** **IA_3** ***S. pastorianus*** **and** ***S. arboricola***. This is an advantageous application because IA_3 *S. pastorianus* and *S. arboricola* had entirely unknown structures and undeveloped methods, thus having the optimized methods for S. Cerevisiae will **streamline further research into them.** Additionally, we have shown that **IA_3** ***S. pastorianus*** **exhibits only α-helices** across the tested TFE volumed and that **IA_3** ***S. arboricola*** **exhibits almost entirely α-helical structures** across TFE volumes, with **more research required to explore these results**. This study is a small part of the larger study into correlating the intrinsic parameters of disordered sequences to the structures and eventually their functional mechanisms. This data **contributes** the **fundamental structural composition of** **IA_3** ***S. pastorianus*** **and** ***S. arboricola*** which allows for **more** in-depth structural **studies** to be **done in the future**.

Future Research

- CD on **IA_3** ***S. pastorianus*** **and** ***S. arboricola mutants***
- CD on **cleaved** IA_3 *S. pastorianus* and *S. arboricola*
- **CW EPR** on IA_3 *S. pastorianus* and *S. arboricola* with **significant labeling sites**
- Collect data on **more IDPs** and **IDRs** in general.

Acknowledgements

My sincerest thanks to **Dr. Gail Fanucci** and **Tianyan Li** for their unwavering support and mentorship throughout this research process. Also, a huge thanks to **Danae Perry**, **Ayan Desai**, **Siddarth Ranganath**, **Rachel Pinskey**, **Mily Gandhi**, and the rest of the SSTP and CPET staff who made this research possible.

Resources

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