A Dehydrin Antifreeze Protein: Protecting Cells at Sub-zero Temperatures

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Summer Summary

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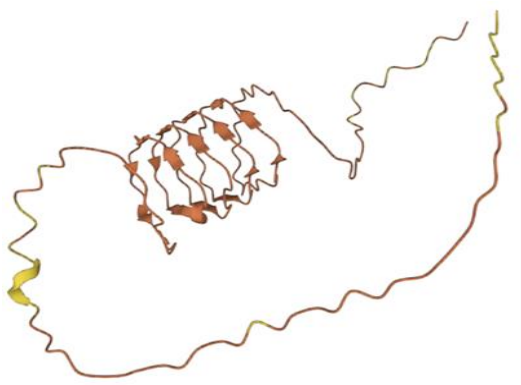
In the past months working in the Varga group, I continued working to characterize the structure and function of an antifreeze protein (AnAFP) from the desert shrub *Ammopiptanthus nanus*. This protein is of particular interest to our group because it falls in the family of proteins known as dehydrins, which help plants survive during periods of water scarcity. Dehydrins bind enzymes and membranes during periods of stress to stabilize cells during dehydration. Previous work in the Varga group by Dr. Katarina Jovic showed that AnAFP can preserve the enzymatic activity of the cold-sensitive enzyme lactate dehydrogenase during cold denaturation. To further understand the cryoprotective capabilities of this protein we had two aims: 1: experimental characterization of the antifreeze properties of AnAFP, and 2: Application of AnAFP in cryopreservation of cells. We investigated the first aim (experimental characterization of the antifreeze properties of AnAFP**)** by performing ice recrystallization inhibition (IRI) activity assays. IRI assays determine if the protein has any effect on the formation of ice crystals by tracking the growth of a monolayer of ice over time. These crystals are imaged on the temperature-controlled microscope stage in the Varga lab. We then analyze the size of the ice crystals using ImageJ analysis software. AnAFP shows negligible IRI activity meaning the mechanism by which it cryoprotects is not ice recrystallization inhibition. While these are preliminary results, we are still in the process of collecting data on additional concentrations of AnAFP.

(A)



(B)

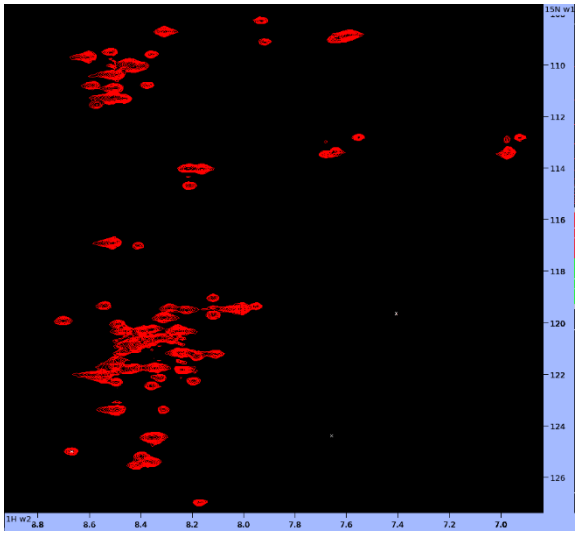


Figure 1. Title. (A) AlphaFold predicted structure of AnAFP, red regions indicate low confidence. (B) ^{1}H-^{15}N HSQC NMR spectrum of AnAFP, in which each peak corresponds to one amino acid in the protein backbone or sidechain.

The second aim of our study (application of AnAFP in cryopreservation of cells) was delayed because we moved our cell culture room from Kendall Hall to Rudman Hall. In place of this we had the goal to elucidate the structure of the protein by using nuclear magnetic resonance (NMR). Making NMR samples consists of expressing the protein with *E. coli* and then purifying the bacterial cell lysate with affinity chromatography. To begin our study, we began by optimizing

the conditions in which the protein would remain soluble for long enough to acquire an NMR spectrum. We determined appropriate buffer conditions for NMR by buffer exchanging the protein into different conditions and tracking protein solubility via UV-Vis spectrophotometry. Once we settled on appropriate buffer conditions, we collected NMR spectra at different temperatures to further increase the signal/noise ratio. Figure 1 shows an example of an ^{1}H-^{15}N Heteronuclear Single Quantum Coherence (HSQC) spectrum that we acquired using a 700 MHz NMR spectrometer in August at 10 ºC. Collecting spectra at lower temperatures yielded better results because of the disordered and highly flexible regions of AnAFP. We are still in the process of acquiring NMR spectra to optimize the conditions for AnAFP.

Overall, we have successfully characterized the IRI activity of AnAFP, and we are continuing to perform NMR experiments to determine the structure of the protein. In the coming weeks our cell culture laboratory space will be fully operational in its new space, and once this is complete, we will begin performing cryopreservation studies with AnAFP.