Using Liquid-Observed Vapor Exchange (LOVE) NMR to Identify Motifs in Protein Dehydration Protection

Understanding factors that affect dry protein structure and stability has become a focus of the pharmaceutical industry as protein-based drug (PBD) formulations have become increasingly common. While drying these formulations can increase thermostability and shelf life, it may also cause denaturation or aggregation of the PBD. Stabilizers can be added to drug formulations to discourage misfolding during dehydration and help increase protein stability in the dry state; however, their mechanisms of action remain poorly understood. Liquid-Observed Vapor Exchange (LOVE) NMR is a solution NMR-based method that provides residue-level information about the structure of dehydrated proteins and the impact of protectants thereon. Using this method, I highlight similarities is stability enhancement of the model protein GB1 using protectants that are effective at protecting proteins from dehydration-induced damage. My focus is understanding protection patterns of the cytosolic abundant heat-soluble protein D (CAHS D), a desiccation protectant discovered in the tardigrade (H. dujardini), which is known to form gels and is believed to form glasses. I compare the protection profile of CAHS D to gelatin (collagen) which forms gels and to hydroxyectoine which forms glasses and discuss protection patterns.