What's Linker Have to Do with it? Examining the Structure & Stability of Palladin's Ig3-4 Linker Region

Lauren Hughes, Nathan Ta, Jacquelyn Martinez-Landa, Rachel Sargent, Moriah R. Beck

Department of Chemistry and Biochemistry, Wichita State University

The protein actin is integral to movement and cytoskeleton function within the human body. Actin participates in more protein-protein interactions than any other known protein; one such relationship involves palladin. Palladin is comprised of five immunoglobulin-like domains (Ig), each connected via an unstructured linker region. Previous research has proven that Ig3 is the minimal actin-binding domain, however, binding affinity is significantly increased when the Ig3-4 linker domain is present. To examine the effects of this Ig3-4 linker on overall actin binding, the Beck lab introduced several mutations. When ten arginines within Ig3-4 were converted to alanine, the binding ability of both actin and palladin was completely disrupted. Our current research seeks to determine how mutations within this Ig3-4 linker region affect the structure and stability of palladin. Purified wild-type and mutated linker proteins were examined using circular dichroism (CD) spectroscopy, where wavelength scans of 185 to 260 nanometers were used to determine the secondary structure. The proteins also experienced thermal denaturation conditions of 20°C to 90°C, which indicated their stability. An initial analysis revealed slight differences in the stability and basic structural components of wild-type and mutated palladin. The scans were then processed using DichroWeb software, which creates charts detailing folding, structure, and wavelength impacts. A preliminary review of this data indicates that the structure of mutated Ig3-4 linker contains more beta-sheet folds, signifying that the linker region is developing despite diminishing binding in both actin and palladin. Future examinations will involve mechanical testing, extended protein comparisons, and NMR spectroscopy.