Poster 4

Stability and Equilibrium Unfolding Pathway of Single Chain Monellin

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Single chain-monellin (SCM) was engineered protein by fusing the two chains of monellin. From the view of protein folding, SCM serves as an ideal model system due to the following reasons: First, it consists of simple distinct structural elements (α-helix and β-sheet) which are assembled in a perpendicular manner. Second, it has the unique fluorescent probe of Trp3. Concerned with the folding intermediate, we examined the equilibrium unfolding pathways of SCM induced by GdnHCl and pH using various spectroscopic techniques (fluorescence, circular dichroism, and nuclear magnetic resonance) as well as gel filtration chromatography. Through the noncoincidence of the transition curves monitored by fluorescence and far-UV CD, the presence of the stable unfolding intermediate was showed around 1.5 M [GdnHCl]. Gel filtration chromatography revealed that this state was a monomeric form that is distinguished by the slightly increased hydrodynamic volume from the native state. NMR spectroscopy indicates that the overall conformation at 1.5 M [GdnHCl] is very native-like, where the slight differences in the chemical shift from the native state are shown in both terminus and loop region. Furthermore, based on our studies, we propose that not entire β-sheet domain but each polypeptide segment on SCM which correspond to A and B chain in monellin acts as an cooperative folding unit.