A-9

Characterization of the Unfolding Intermediate State and Equilibrium Unfolding Pathway of Single Chain Monellin

Heedouk Hong*, Yoon-hui Sung, Yong-Rok Kim[1], and Weontae Lee
Dept. of Biochemistry Yonsei University and [1]Dept. of Chemistry, Yonsei University

Single chain-monellin (SCM) was recently constructed by fusing the two chains of monellin. From the view of protein folding, SCM serves as an ideal model system especially in tackling α-helix-β-sheet interactions 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. From 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]. Furthermore, neither entire β-sheet domain nor each polypeptide segment on SCM which correspond to A and B chain in monellin was identified to act as an cooperative folding unit. Gel filtration chromatography revealed that this state was in 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. Therefore, we propose that the folding mechanism of SCM can be viewed under the modular assembly model.