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Engineering a Non-Inhibitory Serpin, Ovalbumin

Yeon-Hee Jeoung* and Myeong-Hee Yu
Division of Protein Engineering, Korea Research Institute of Bioscience and Biotechnology, P. O. Box 115, Yusong, Taejon 305-600, Korea

Serpins (serine protease inhibitor) are single polypeptide proteins of around 400 amino acids, and have a conserved secondary structure consisted of three β-sheets and nine α-helices. Native conformation of inhibitory serpins is a metastable and requires conformational changes to inhibit target protease. While ovalbumin, a member of non-inhibitory serpin superfamily, very thermodynamically stable native conformation. Unlike inhibitory serpins, ovalbumin does not spontaneously insert the reaction site loop (RSL) into β-sheet A upon cleavage by protease. In the inhibitory serpin, RSL has a conserved small uncharged side chain and, particularly P14, and P12-P10 of hinge region are known to be important in the insertion of RSL.

In order to test whether changes in this region alone can transform ovalbumin into a proteinase inhibitor with strained native conformation, we substituted amino acids in the hinge region. Native conformations of several mutant ovalbumin molecules were become unstable compare to wild-type ovalbumin. When these ovalbumin mutants were cleaved by elastase, RSL were able to be for conformational changes inserted into β-sheet A, presumably by lowering the transition energy barrier.