C-2

Elucidation of Serpin's Conformational Switch Mechanism By Rapid Kinetic Study

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The native form of serpin (serine protease inhibitor) is kinetically trapped in metastable state. Metastability in these proteins is critical to their biological function. Serpins inhibit target proteases by forming a stable covalent complex in which the cleaved reactive site loop of the serpin is inserted into β-sheet A of the serpin with concomitant translocation of the protease to the opposite of the initial binding site. Despite recent determination of the crystal structures of a Michaelis protease-serpin complex as well as a stable covalent complex, details on the kinetic mechanism remain unsolved. In this study we constructed several α_1-antitrypsin variants and examined their kinetic mechanism of loop translocation and formation of protease-serpin complex by stopped-flow experiments of fluorescence resonance energy transfer as well as quenched-flow experiment. We report here the relationship of serpin's conformational switch mechanism with Inhibitory activity. There is little direct correlation between loop insertion rate and inhibitory activity. Rather, disrupting a salt bridge between R196 and E354 accelerates loop translocation even though it impairs the inhibitory activity. Moreover, the serpin's reactive site loop is translocated, at least partially, prior to loop cleavage.