A-11

Regulation of the Inhibitory Function of α_1-Antitrypsin by Native Metastability

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The native forms of some proteins such as inhibitory serpins (serine protease inhibitors) and viral membrane fusion proteins are metastable, which is critical to their functions. To understand the mechanism of how native metastability regulates the inhibitory function of serpins, we characterized stabilizing mutations of α_1-antitrypsin, a prototype serpin, in which Gly 117 was replaced by a series of larger hydrophobic residues. Conformational stability of the α_1-antitrypsin variants increased linearly as the van der Waals volume of the side-chain at 117 increased, suggesting that a cavity exists in the region. The net increase of stabilization energy was 35 cal/mol/Å^3, corresponding to 0.87 kcal/mol per a single methylene group. The activity analyses of the variants against a target elastase showed that there is a strong inverse correlation between the conformational stability and the inhibitory activity. Stopped-flow measurement during the interaction with a target protease showed that the stabilizing mutations of α_1-antitrypsin retarded the conformational switch in which a massive rearrangement of the major β-sheet occurs including the insertion of the reactive center loop as a strand. The correlation between the extent of stability increase and the decrease in the rate of the conformational switch provides direct experimental evidence that metastability due to the structural defect of inhibitory serpins regulates the inhibitory function by facilitating the conformational switch needed for complex formation.