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Functional Role of the Native Strain that is Distributed throughout an α_1-antitrypsin

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The native strain of serpins (serine protease inhibitors) has been recognized as a mechanism of biological regulation. Indeed, some stabilizing single residue mutations of human α_1-antitrypsin, a prototype serpin, relieved local strain and caused the loss of inhibitory activity. The native strain of α_1-antitrypsin is distributed throughout the whole molecule, but the strain that regulates the function directly is highly localized in the regions that appear to be mobilized during complex formation with a target protease. This raised the question why the strain is distributed over the whole molecule. To address this point, we increased the stability of α_1-antitrypsin greatly via combining various amino acid substitutions scattered over the molecule that did not affect the activity. The results showed that that a substantial stability increase (over 13 kcal mol^{-1}) affected the inhibitory activity with an excellent correlation of about 11% activity loss per kcal mol^{-1}. However, additive stability increase of the activity-affecting single residue substitutions in the loop insertion region by these multiple stable mutations did not cause a further activity decrease, indicating that the step regulated by single activity-affecting substitutions governs the complex formation predominantly. The results strongly suggest that, the structural design of the native strain for functional regulation is limited to the loop insertion region and the strain scattered over the α_1-antitrypsin molecule regulates the inhibitory activity not by the designed strain per se but by global stability in general.