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CHARACTERIZATION OF A HUMAN α1-ANTITRYPSIN VARIANT THAT IS AS STABLE AS OVALBUMIN BUT RETAINS INHIBITORY ACTIVITY

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The metastable native state of proteins plays an important role in regulating biological functions. The native strain of serpins (serine protease inhibitors) are considered to be crucial for the inhibitory function. Several thermostable mutations of human α_1-antitrypsin, a prototype inhibitory serpin, were identified in a systematic search targeted at the hydrophobic core of the molecule [*Nature structural biology*, vol. 3, no. 6, 497-500 (1996)]. The mutations stabilized the native state of the molecule, and retarded the conversion into the more stable latent form. Combination of seven such stable single amino acid substitutions (Multi-7) of α1-antitrypsin increased the midpoint of unfolding transition to almost that of ovalbumin, a non-inhibitory but more stable serpin. The Multi-7 α1-antitrypsin, however, showed the normal inhibitory activity. In an equilibrium folding-unfolding transition the wild type α1-antitrypsin exhibited a stable intermediate, but the Multi-7 molecule did not show the intermediate. In addition, the unfolding transition of Multi-7 was not cooperative, as seen in regular globular proteins, but was rather abrupt. The Multi-7 α1-antitrypsin molecule converted into the latent form much less readily, but underwent a conformational switch into a stable relaxed form upon cleavage at the reactive center loop, indicating that the mutant molecule still retains the native strain. These results suggest that the Multi-7 α1-antitrypsin is a more stable but poorly folded form that is approaching the state of an alternative energy minimum rather than the global minimum.