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FOLDING-UNFOLDING KINETICS OF HUMAN α_1-ANTITRYPSIN: CHARACTERIZATION OF A KINETIC INTERMEDIATE THAT IS BRANCHED TO THE NATIVE AND AGGREGATION FORM

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Aggregation of human α_1-antitrypsin (α_1-AT) during folding occurs both *in vitro* and *in vivo*. *In vivo* aggregates of mutant α_1-AT such as M_{malton} (Phe52 deleted) and Z (Glu342 $\rightarrow$ Lys) variants have pathological consequences. In order to analyze the process of α_1-AT aggregation in detail, the folding-unfolding kinetics of α_1-AT was examined by monitoring intrinsic Trp fluorescence and ANS binding. While the unfolding of α_1-AT shows a single exponetial phase, refolding shows three exponential phases, as revealed by the fluorescence signal from Trp194. The fast phase ($\tau_{1,r}$ = 40 sec) was independent of urea concentration, which may be the *cis-trans* isomerization of prolyl residue. The medium phase ($\tau_{2,r}$ = 200 sec) yielded an intermediate (I_N), that is capable of elastase binding. The slowest ($\tau_{3,r}$ = 1000 sec) phase completes the refolding to the native protein, which intersects with the unfolding kinetics at the same urea concentration (1.9 M) as the equilibrium midpoint. The changes monitored by extrinsic ANS fluorescence during the transitions were similar to those monitored by intrinsic Trp fluorescence. Various experimental data suggest that it is the I_N that is prone to the kinetic competition between the on-pathway to native protein and the aggregation.