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Effect of Single Amino Acid Replacements on the Folding of α_1-Antitrypsin

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The effect of stabilizing single amino acid replacements at the sites of Phe 51, Ala 70, and Met 374 on the folding of α_1-antitrypsin was investigated by fluorescence spectroscopy. The residues Phe 51 and Met 374 are located in the hydrophobic core of the molecule, B β-sheet. Substitutions of Phe 51 by Leu, Ile, or Cys retarded the unfolding without significant effect on the refolding rate in guanidine-mediated folding-unfolding transition. Substitutions of Met 374 to Ile or Val also retarded the unfolding, but they increased the refolding rate also. Analyses of the free energy change of the transition state with respect to the change in the overall free energy of folding imply that interactions in the hydrophobic core are formed earlier at the Met 374 site than at the Phe 51 site. Substitution of Ala 70 by Gly, which is located at the exposed loop prior to C helix, stabilized both the native state and the transition state with respect to the unfolded state. Interestingly, the substitution by Asp increased the unfolding rate without affecting the stability of the native form significantly. These results imply that the residue 70 is accessible to surrounding solvent in the transition state as in the native state, and the stabilizing effect of charged side-chain is only manifested in the transition state.