

## MA-3

### Mutations in the Reactive Center Loop of $\alpha_1$ -Antitrypsin That Retard the Loop Insertion

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$\alpha_1$ -Antitrypsin is a key plasma serine protease inhibitor and its prime physiological role is an inhibitor of leukocyte elastase. The reactive center loop of  $\alpha_1$ -antitrypsin is characterized by the unusual mobility and the ability of insertion into the central  $\beta$  sheet A. In particular the rate of loop insertion is considered to be critical for the formation of a stable complex between a serpin and its target protease. We have screened for thermostable mutations in the reactive center loop including the strand 4 of A sheet (s4A) and the strand 1 of C sheet (s1C), and have evaluated the effect of substitution at positions, P1, P14, and P10' on the inhibitory activity. The stoichiometry of inhibition (S.I.) values were greater than 1 for these  $\alpha_1$ -antitrypsin variants from 2 to 70. The analyses of inhibitor-protease complex formation on the SDS-polyacrylamide gel electrophoresis showed that these mutations caused to partition more variant molecules into the substrate pathway than the wild type does. It is likely that stabilizing mutations in reactive center loop of  $\alpha_1$ -antitrypsin have reduced inhibitory activities due to the retardation of the loop insertion.