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Probing the movement of helix F region and the stepwise insertion of reactive site loop in α 1-Antitrypsin variants

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α1-Antityrpsin is a member of the serine protease inhibitor (SERPIN) family that shares a common tertiary structure. The reactive site loop (RSL) of serpins is exposed at one end of the molecule for protease binding. Upon cleavage by a target protease, the RSL is inserted into the major β-sheet A, which is a necessary process for formation of a tight inhibitory complex. Various biochemical and structural studies suggest that the rate of the RSL insertion upon binding a target protease is critical for inhibitory activity, and it is thought that helix F region (thFs3A and helix F) located in front of β-sheet A, should be lifted for the loop insertion during complex formation. To understand the details of RSL insertion, Fluorescence resonance energy transfer experiments were carried out by labeling of 7-nitrobenz-2-oxa-1,3-diazol-4-yl(NBD) derivative on each of A347C or A355C position on the background of some activity-affecting stabilized variants (G117F, K335V, near helix F) and of A350P variant (hinge core on RSL); A347C-NBD labeling site was chosen for measuring the insertion rate of distal part of RSL from the hinge core, while A355C-NBD labeling site was chosen for measuring the overall RSL insertion rate. We also engineered disulfide bond at several sites between helix F region and β-sheet A for locking of its movement, during the RSL insertion. A significant activity loss was not found by locking of helix F to β-sheet 3A. The insertion rate of distal region of RSL monitored by A347C-NBD was almost the same for all throughout the proteins, while the overall insertion monitored by A355C-NBD was retarded for the variants we chose. The results suggest that there is little correlation between inhibitory activity and the relative movement of helix F against β-sheet 3A, but imply that the helix F region and hinge core (A347-A350) may affect the full insertion of RSL by modulating relative movement of its proximal part, thereby control the inhibitory activity of serpins.