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Determination of Translocation and Deacylation Rate Constants for Complex Formation between Serpin and Protease

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Serpins inhibit target proteases by forming tight acyl complex via incorporation of the reactive center loop into β-sheet A. Metastability of the serpins control the translocation of the protease from the initial binding site to the opposite pole of the serpin. Recently the crystal structure of a serpin-protease complex revealed that the active site of the protease is distorted. However, controversies on kinetic mechanism remain mainly due to difficulties in kinetic measurement of acylation (k_{ac}), translocation (k_{tr}), and deacylation (k_{deac}) steps. To address the problem, we analyzed stopped-flow kinetics of fluorescence energy transfer from the tryptophans in trypsin to a dansyl group labeled at Cys^{314} of α_1-antitrypsin during complex formation. Under pseudo-first order reaction conditions with protease in excess, time-resolved fluorescence change between trypsin and α_1-antitrypsin showed a double exponential growth function. The pH-dependence of each phase revealed that fast and slow phases correspond to acylation (k_{ac}=31.2 s^{-1}) and translocation (k_{tr}=1.76 s^{-1}) steps, respectively. Deacylation rate constant was determined to be 0.08 s^{-1}. Compared to hydrolysis kinetics of octapeptide (RCL of α_1-AT from P_4 to P_4'), these results suggest that acylation is facilitated whereas deacylation is strongly suppressed.