

P009 Protein domains regulating the potency of therapeutic thrombolytic proteases

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Plasminogen activators are used as thrombolytic agents to generate plasmin, a serine protease capable of digesting fibrin and lysing blood clots. Tissue plasminogen activator (tPA) and the proenzyme plasminogen (Pgn) are multi-domain proteins that bind to fibrin and regulation of plasmin generation is dependent on the assembly of tPA-Pgn-Fibrin ternary complexes. We have investigated the kinetics of Pgn activation in fibrin using an accurate assay system to derive apparent K_m and k_{cat} values for tPA domain variants generated in an insect cell expression system. Native tPA is composed of 5 domains (F-G-K1-K2-P) and fibrin binding is initially via the F (finger) domain, and later, as fibrin is degraded, by the K2 (Kringle 2) domain in a positive feedback mechanism. Amidolytic assays indicate some domain interactions affecting the protease domain (P) present in tPA and K1tPA (FGK1K1P) not present in rPA (K2P). In Pgn activation assays in fibrin, rPA binds poorly to native fibrin but plasmin generation accelerates indicating the importance of the K2 domain, such that rPA activity improves from 5 to 15% of the activity of tPA as fibrin digestion progresses; while K1tPA remains at between 18-22% of tPA activity. Analysis of app K_m and k_{cat} values indicates that increased Pgn binding to partially digested fibrin accounts for 2-3 fold stimulation of plasmin generation by tPA and K1tPA regardless of the presence of the K2 domain.