Molecular Mechanisms of Thrombin-Mediated Fibrin Assembly

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Conversion of fibrinogen into fibrin (fibrin assembly) is mediated by thrombin, which cleaves two pairs of fibrinopeptides, fpA and fpB, from fibrinogen Aα and Bβ chains, respectively. Fibrin assembly occurs in two stages starting with the removal of fpA, which triggers formation of two-stranded protofibrils, followed by the removal of fpB, which enhances lateral association of protofibrils into thicker fibers. Non-substrate interaction of thrombin with the central part of fibrin(ogen) seems to play an important role in these processes. To establish the structural basis for the sequential cleavage of fpA and fpB, we crystallized thrombin in complex with a fragment corresponding to the central part of fibrin(ogen), and solved the structure of this complex at 3.65 Å resolution [1]. Next, we modeled possible arrangements of fibrinopeptides-containing NH_2-terminal portions of the Aα and Bβ chains, which were not identified in the electron density map. The resulting structure reveals that thrombin binds fibrinogen in a way that promotes positioning of the Aα chains in its active site cleft. This explains the preferential removal of fpA at the first stage of fibrin assembly. Subsequent modeling of a protofibril by docking the structure of the dimeric fibrin-derived D-D fragment into the complex suggests that the fpB-containing portions in such structure should interact with D-D to restrict their positions to the vicinity of the active site cleft. In agreement, surface plasmon resonance experiments reveal that these portions indeed interact with D-D. These provide the rationale for the accelerated removal of fpB upon the formation of protofibrils.

[1] Pechik I., Madrazo J., Mosesson M.W., Henandez I., Gilliland G.L., Medved L., *Proc. Nat. Acad. Sci. USA*, 2004, **101**, 2718.

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