Specific proteolytic cleavage of reelin by coagulation factor X

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Reelin is an extracellular glycoprotein that was first identified in mice with reeling gait. Despite that three different cleavage forms of reelin (420 kDa, 310 kDa, and 180 kDa) have been demonstrated to present in plasma and act as a positive regulator of platelet spreading on fibrinogen, the protease that is responsible for generating reelin cleavage products are still elusive. To identify the protease that is responsible for generating form-specific reelin, bioinformatic analysis using the PeptideCutter program revealed that the coagulation factor X (FX) is a potential reelin protease. *In vitro* cleavage assay demonstrated that the active form of FX (FXa) executed the cleavage of 420 kDa full-length recombinant reelin into the 310 kDa and 110 kDa immunoreactive fragments. The FXa-mediated cleavage of reelin was inhibited by the serine protease inhibitors leupeptin and phenylmethanesulfonylfluoride, EDTA, and the FXa-specific inhibiting peptide Tenstop, implicating that the calcium-dependent serine protease activity of FXa is required for cleavage of reelin at the C-terminus. In vitro transcription/translation of various reelin deletion mutants followed by FXa cleavage assay facilitated the identification of FXa cleavage site which was located between the amino acids 2315 and 2568. Site-directed mutagenesis further identified arginine 2695 as a critical recognition site for FXa-mediated reelin cleavage. The binding of the recombinant 310 kDa proteolytic reelin fragment toward platelets was significantly reduced when compared with the full-length 420 kDa reelin, implicating that the C-terminus of reelin is important for platelet binding. Taken together, we report for the first time that FX is a protease that is responsible for C-terminus reelin cleavage leading to a decrease in reelin-platelet interactions. This study thereby provides a biochemical basis for the interplay between reelin and haemostasis.