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AN INVESTIGATION INTO THE STRUCTURAL FEATURES THAT CONTROL FACTOR XIII STABILITY AND SUBSTRATE SPECIFICITY

ABSTRACT:

Factor XIII (FXIII) is a transglutaminase enzyme with multiple physiological roles that is found in plasma and cells of bone marrow origin.[1] The catalytic A subunit (FXIII-A) is made of an N-terminal activation peptide (AP), β-sandwich, catalytic core, and two β-barrel domains.[1] FXIII crosslinks the sidechains of glutamine (Q) and lysine (K) residues across plasmatic and cellular substrates.[2] Because of its involvement in clot stabilization and determining fibrin clot size, FXIII-A is regarded as a target for developing new anticoagulants with minimal bleeding risks.[3] However, the FXIII-A structural features that control its stability and substrate specificity largely remain unknown.

Plasma FXIII is activated by thrombin-mediated AP cleavage in the presence of Ca^{2+} to form FXIII-A*, whereas cellular FXIII is non-proteolytically activated to FXIII-A° with Ca^{2+}.[1] FXIII-A* is more conformationally heterogeneous than FXIII-A°.[4] Crosslinking of a fluorescent K-mimic to a series of FXIII substrates indicated that the exposure of a secondary substrate binding site and protein substrate disorder determine the substrate specificity of FXIII-A*/A°.[5] The AP cleft in the β-sandwich domain of FXIII-A* was proposed as a substrate binding site, and the residues K156, F157, R158, R171, and R174 were identified to be part of a recognition site for substrates, including Fibrinogen (Fbg)-α chain, Fbg-γ chain, and actin.[6] Mutations of these FXIII residues significantly reduced but did not abolish the FXIII crosslinking activity.[6] This result and previous studies suggested the existence of additional substrate binding sites closer to the FXIII active site. Mutations of FXIII catalytic core residues exposed during activation indicated that residues W315, Y214, and Y227 are also involved in binding substrates.

The FXIII activation peptide (AP) is a unique feature that distinguishes it from other transglutaminases and protects the enzyme from auto-activation and unwanted degradation.[7] By site-directed mutagenesis of FXIII-A AP residues and crosslinking assays, the AP residues P36, P27, and N20S were found to be critical for thrombin-mediated AP cleavage. Additionally, the degradation of E23 and D24 mutations demonstrated their importance in FXIII-A_2 stability. The effects of AP mutations on FXIII transglutaminase activity suggested protein conformational changes and less accessibility to the catalytic site.

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