Extended D-E interactions near the classical knob-hole binding site play an important role in fibrin polymerisation and clot stability


1 Thrombosis and Tissue Repair Group; Division of Cardiovascular and Diabetes Research; Leeds Institute of Cardiovascular and Metabolic Medicine; University of Leeds; UK
2 Institute of Molecular Medicine, University of Lisbon, Lisbon, Portugal
3 Molecular and Nanoscale Physics group, School of Physics and Astronomy, University of Leeds, UK
4 Department of Chemistry, University of Massachusetts, Lowell, Massachusetts, USA

BACKGROUND: Previous studies indicate the presence of an extended binding interface beyond the traditional knob-hole interactions that occur when thrombin converts fibrinogen to fibrin (Kononova, JBC 2013): γAsp297, γGlu323 and γLys356 in the D-region interact with βLys58, βAsp61 and βHis67 in the E-region from another adjacent fibrin monomer.

AIMS: To investigate the role of the extended knob-hole interface in polymerisation kinetics, clot structure and mechanics.

METHODS: Four recombinant human fibrinogen variants and WT were produced: γDEK (γD297N/E323Q/K356Q) with mutations in all γ-chain residues involved in extended knob-hole binding, and variants with single mutations at γD297N, γE323Q or γK356Q.

RESULTS: Maximum OD for all variants was reduced compared to WT, whereas lag phase was only extended for γDEK. γDEK was more readily deformable (loss tangent, tanδ), but single mutant variants were unchanged compared to WT. Electron microscopy showed γDEK forming the densest clots, followed by the single mutants, then WT. Fibers for all variants were thinner than WT, with γDEK being the thinnest. Variants γDEK and γE323Q produced a denser clot network in hydrated conditions, whereas γD297N and γK356Q were similar to WT, and all were slower to lyse, except γD297N. There were no differences in clot size between γD297N and WT fibrinogen in an in vivo model of murine femoral thrombosis.

CONCLUSIONS: The abolition of electrostatic interactions responsible for extended binding results in altered polymerisation kinetics, clot structure and viscoelastic properties. Our findings demonstrate that the D-E binding interface extends beyond the classical knob-hole interaction to reinforce fibrin polymerisation.

I, Nathan Asquith would like to apply for the Outstanding Abstract Award.

Corresponding Author: Professor Robert Ariëns: Division of Cardiovascular and Diabetes Research LIGHT Laboratories Clarendon Way University of Leeds Leeds, LS2 9JT T +44 (0) 113 343 7734 F +44 (0) 113 343 7738 E r.a.s.ariens@leeds.ac.uk