

Supplementary Information

Evaluating the Effects of Fibrinogen α C Mutations on the Ability of Factor XIII to Crosslink the Reactive α C Glutamines (Q237, Q328, Q366)

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Supplementary Table S1:

Primers used to introduce single amino acid substitutions into Fibrinogen α C (233-425)

Mutation	Forward Primer 5'-3'	Reverse Primer 5'-3'
E396A	CTGGGGCACATTTGAAGCGGT GAGGAAATG	CATTTCTGACACCGCTTCA AATGTGCCCCAGTCTGG
Q237N	GGATCCACAGACATGCCGAAC ATGAGTGGAAGGCATTAACAG	ATGCCTTCCACTCATGTTTCG GCATGTCTGTGGATCC

Supplementary Table S2:

Sequence and mass of proteolytically derived peptides containing α C Q237, Q328, and Q366

Reactive Glutamine	Amino Acid Sequence	Theor m/z	Exptl m/z	Digest
Q237 ^a	<i>GPLGSTDMPQMRME</i>	1549.7	1550	GluC
Q237N	<i>GPLGSTDMPNMRME</i>	1535.5	1535	Gluc
Q328	<i>NSGSSGTGSTGNQNPGRPRP GSTGTW</i>	2448.1	2448	Chymotrypsin
Q366	<i>SSVSGSTGQWHSE</i>	1348.6	1349	GluC
E396	<i>SGSFRPDSPGSGNARPNNPDWGTFEE</i>	2778.2	2778	GluC
E396A ^b	<i>SGSFRPDSPGSGNARPNNPDWGTFE</i>	2649	2648	GluC

^aMS/MS analysis on the peptide containing Q237 revealed that amino acids GPLGS are located just N-terminal to the α C 233-425. These amino acids remain following PreScission (human rhinovirus 3C) protease cleavage of the GST- α C (233-425). The sequences containing Q328 and Q366 were readily identified using a theoretical protease digest (Protein Prospector UCSF).

^bWith the E396A mutation, the GluC cleavage site shifts from E396 to E395.

Supplementary Table S3:

Average retention times and m/z values for peptide fragments containing reactive glutamines analyzed by LC-MS

Reactive Glutamine monitored	Average retention time (min)	$m/z +1$	$m/z +2$	$m/z +3$
Q237	19.4	1549.6807	775.3440	517.2318
Q237-GEE	22.1	1635.7175	818.3624	545.9107
Q328	12.7	2448.0770	1224.5422	816.6972
Q328-GEE	18.3	2534.1138	1267.5606	845.3761
Q366	9.7	1348.5764	674.7918	450.1970
Q366-GEE	13.5	1434.6132	717.8102	478.8759