SUPPORTING INFORMATION

Table S1. γ-chain tryptic peptides: predicted and observed masses for γ–γ dimers excised from SDS-polyacrylamide gels of papain fibrin and (control) thrombin-F13a fibrin.[1]

gamma res	amu pred	papain 1/14/14	papain 4/24/14	papain 5/28/14	F13a 1/14/14	F13a 4/24/14	F13a 5/28/14
6–14	1194.4	1194.4	1194.6	1194.6	na[1]	1194.4	1194.5
15–35	2418.1	2417.2	no pk[2]	2417.3	2418n[3]	2417.1	2417.2
63–85	2521.1	2521n	2521n	no pk	2521n	2521n	2519.4
96–108	1491.9	1491.7	1493.7	1491.9	na	1491.8	1491.8
109–120	1513.7	1514.6	1514.7	1514.8	1514.7	1513.8	1513.8
128–140	1560.7	1560.6	1560.7	1559.9	1560.6	1560.7	1560.8
141–151	1226.5	1226.5	1226.6	no pk	na	1226.6	1226.6
152–159	963.1	na	963.5	1226n	na	1226.6	1226.6
163–173	1293.8	1293.6	1293.6	no pk	na	1291.6	1291.7
174–196	2662.1	no pk	2662.1	2661.4	2662n	no pk	2661.4
207–212	851.1	na	851.4	849.1	na	851.5	851.4
213–232	2207.7	2209.1	2207.1	2209.2	2206.9	2207.1	2207.1
233–247	1683.3	1682.9	1682n	1683.0	1682.9	no pk	1682n
248–256	1117.2	1118.4	1118.5	1117.6	na	1118.5	1118.5
257–266	1134.4	1134.4	1135.6	no pk	na	1134.5	1134.5

276–302	2835.5	no pk	no pk	2834n	2834.1	no pk	no pk
303–321	2291.6	2290.1	2289.2	2293.2	2291.9	2293.2	no pk
322–338	2016.3	2015.8	2016n	2014n	no pk	no pk	no pk
339–356	2012.3	2012.9	2013.0	2014.0	2012.8	2013.0	2013.0
357–373	1894.3	1895.8	no pk	no pk	1895.8	1895.9	1895.9
392–406	1546.1	1545.7	1546.8	1546.8	1545.6	1548.8	1548.8
407–427[4]	2663.7	no pk	2662.1	2662.5	no pk	no pk	2661.4

[1] Only peptides in the mass range 800–3000 shown. In the case of the January papain entries, the mass range was restricted to 1000–3000 and for January FXIIIa series only 1500–3000 (na indicates out of mass range observed).

[2] “no pk” denotes no distinguishable peak or “in the grass.”

[3] Values with no decimal and an “n” attached were unlabelled on the display and were estimated from axis values.

[4] The segment 407–427 pertains only to the γ’ chain (minor form).

Figure S1. Inhibition of papain-catalyzed gelation of human fibrinogen by 0.8 mM GPRPam.

Figure S2. A. SDS gel electrophoresis (non-reducing conditions). Time course of papain digested fibrinogen in presence (even numbered lanes) and absence (odd numbered lanes) of the synthetic peptide knob GPRPam. Note that cross-linked material increasingly accumulates in the stacking gel for those samples without the inhibitor. **B.** SDS gel electrophoresis (reducing conditions). Time course of papain digested fibrinogen in presence (even numbered lanes) and absence (odd numbered lanes) of the synthetic peptide knob GPRPam. Arrow denotes position of γ-γ dimer that does not form in the presence of GPRPam.

Figure S3A. SDS gels (reducing) of six different fibrinogen preparations. A, DEAE peak 1; B, Enzyme Research peak 1; C, GPR-affinity purified; D, recombinant; E, cold alcohol-purified fibrinogen from factor XIII-deficient plasma; "crude", cold alcohol-purified from normal plasma.

Figure S3B. The same six fibrinogen preparations treated with papain to generate "clots." Note that all have bands at the γ-γ dimer position. The left-most lane is untreated

"crude" fibrinogen. (5% acrylamide, SDS gel electrophoresis, reducing conditions).

Figure S3C. The six different human fibrinogen preparations clotted with thrombin in the presence of calcium and cysteine. Only the crude fibrinogen and preparation C (GPR-purified) showed evidence of contaminating factor XIII.

Fig. S4. Sustained digestion of human fibrinogen by papain (fibrinogen 2 mg/ml; papain 10 μg ml). Lanes 2–7 correspond to time points of 5, 10, 20, 30, 60 and 90 min. Lane 1 is the starting fibrinogen, and lane 8 is purified human D-dimer prepared from factor XIIIa cross-linked fibrin. **A.** Unreduced. **B.** Reduced.

Large Scale Preparations of Papain Fibrin

Gel Filtration of NaBr-Dispersable Material.

Approximately 30 mg of plasma-derived fibrinogen was solidly gelled with minimal papain. After 4 hours, the clot was removed, washed, macerated with a Teflon stirring rod and then removed by centrifugation. The protein concentration of the supernatant fluid (clot liquor) was determined by spectrophotometry at 280 nm. Allowing for the fact that the released αC domain is rich in tryptophan and tyrosine, approximately 75% of the original fibrinogen was incorporated into the gel. There was no sign of cross-linked

units in the clot liquor when examined by SDS gel electrophoresis (Figure S2).

The macerated clot was dispersed in 1M NaBr, pH 5.3, to the extent possible, insoluble material amounting to about half the protein being set aside for examination on SDS gels after reduction in the presence of urea and SDS. The soluble material was applied to a BioGel A1.5 column equilibrated with 0.9 M NaBr–0.95 M sodium acetate buffer, pH 5.3 (Figure S5). Aliquots from each of four pools were examined by SDS polyacrylamide gels (Figure S5 inset). There was a clear correlation between fibrin being indispersable in 1M NaBr, pH 5.3, and the abundance of cross-linked material observed on SDS gels. The fraction most soluble in 1M NaBr, pH 5.3, as indicated by its trailing position on the A1.5 column, had the smallest amount of γ-γ, and the totally insoluble material exhibited the most.

Figure S5. Gel filtration of NaBr–soluble material extracted from papain–fibrin. Bio–Gel A1.5 column (2.5 x 44 cms) equibrated with 0.9 M NaBr–0.05 M Na acetate, pH 5.3. Inserts: SDS gels, left, unreduced, right, reduced) earlier peaks contained more cross–linked material than later ones and peak IV contained hardly any at all. Peak V corresponded to small molecular weight material that was not further characterized.

Reduction and alkylation of papain fibrin. A substantial amount of recombinant human fibrinogen (30 mg) was treated with papain, after which the clot was removed, washed well with saline and then dissolved in guanidinium chloride, pH 8.3. The preparation was reduced with dithiothreitol and, after 1 hour, alkylated with iodoacetamide. The preparation was passed over a Sephadex G-150 column (2.5 x 70) in an effort to purify γ-γ dimers and other γ-chain polymers (Figure S6).

Figure S6. Gel filtration on Sephadex G-150 of reduced and alkylated papain-fibrin (2.5 x 44 cms) equibrated with 4M guanadinium chloride, 0.1M Tris, pH 8.3.

Figure S7. Phylogenetic tree of selected cysteinyl proteases. The enzymes denoted by asterisk (*) are reported to gel fibringen. Human cathepsin H degrades fibrinogen in a manner similar to papain but without gelation (37). A similar situation was found for human cathepsin S (this report).