SUPPLEMENTAL DATA

FIGURE S1. **MS/MS and C-terminal amino acid sequence analysis.** The prominent fragment of hFibrinogen's α chain that formed after this plasma protein was incubated with a recombinant hTryptase- β -heparin complex for 1 min (75:1 enzyme:substrate ratio) was purified by SDS-PAGE, and then subjected to exhaustive digestion, LC-MS/MS, and C-terminal amino acid sequence determination. Shown are the susceptible peptides that were identified in the analyses. Although the entire fragment was mapped, only the region C terminal to residue 481 is shown for clarity. Twenty-two spectra were acquired whose C-terminal sequences terminate at Lys⁵⁷⁵, an amino acid that agrees with the specificity of hTryptase- β but orthogonal to the specificity of the enzymes used in the mapping assay. In addition, MS/MS spectral counts diminish significantly C terminal to Lys⁵⁷⁵. We expect these to be due to co-isolated minor degradation products or incomplete cleavage of hFibrinogen by the hTryptase- β -heparin complex. The vertical red line highlights Lys⁵⁷⁵, which is the preferred site of cleavage by the hTryptase- β -heparin complex used in the study.

FIGURE S2. **MS/MS analysis of a 7-kDa peptide in the digest of hFibrinogen**. hFibrinogen was incubated with a recombinant hTryptase- β -heparin complex. The resulting peptides in the digest were subjected to spin-column filtration, trichloroacetic acid precipitation, and SDS-PAGE. The obtained 7-kDa peptide was exhaustively digested with trypsin, and the resulting peptides subjected to MS/MS analysis. Ten peptides were identified, seven of which had different sequences. The identification of these peptides are shown, as well as their location in native α chain of hFibrinogen (GenBank accession # NP_068657). Nine of the ten peptides reside in the 69-mer C terminus of the protein's α chain as expected.

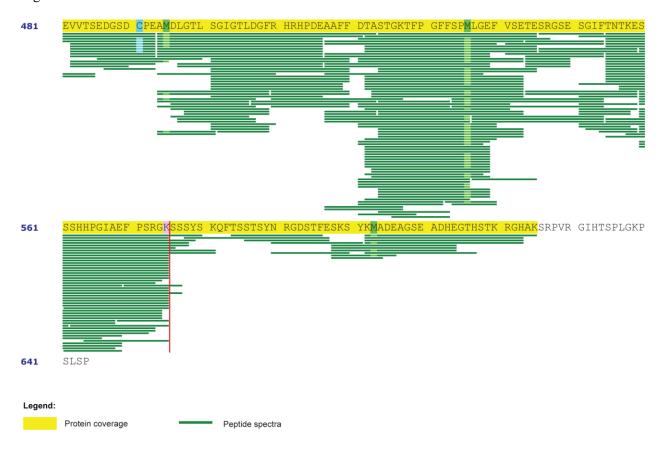


Figure S1

Figure S2

Amino acid sequence of hFibrinogen mfsmrivclvlsvvqtawtadsqeqdflaeqqqvrqprvverhqsackdsdwpfcsdedwnykc psgcrmkglidevnqdftnrinklknslfeyqknnkdshslttnimeilrgdfssannrdntyn rvsedlrsrievlkrkviekvqhiqllqknvraqlvdmkrlevdidikirscrgscsralarev dlkdyedqqkqleqviakdllpsrdrqhlplikmkpvpdlvpgnfksqlqkvppewkaltdmpq (1) *mrmelerpggneitrggstsygtgsetesprnpssagswnsgssgpgstgnrnpgssgtggtat wkpgssgpgstgswnsgssgtgstgnqnpgsprpgstgtwnpgssergsaghwtsessvsgstg qwhsesgsfrpdspgsgnarpnnpdwgtfeevsgnvspgtrreyhteklvtskgdkelrtgkek vtsgsttttrrscsktvtktvigpdghkevtkevvtsedgsdcpeamdlgtlsgigtldgfrhrK⁵⁷⁵ hpdeaaffdtastgktfpgffspmlgefvsetesrgsesgiftntkessshhpgiaefpsrgKs (1) ** (3) (1) * * (2)(2) * ssyskqftsstsynrqdstfesksykmadeaqseadheqthstkrqhaksrpvrqihtsplqkp slsp

Peptides identified by MS/MS analysis of the 7-kDa fragment of hFibrinogen formed when this protein encounters hTryptase- β

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(r) gdstfesk (2)
(r) gdstfesksyk (1)
(r) ggstsygtgsetespr (1)
(k) m*adeagseadhegthstk (2)
(k) qftsstsynrgdstfesk (2)
(n) rgdstfesk (1)
(k) sykm*adeagseadhegthstk (1)
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