Supporting information

Molecular interaction site on procoagulant myosin for factor Xa-dependent prothrombin activation

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SUPPLEMENTAL MATERIALS AND METHODS

Materials

Human factors Xa and Va were from Hematologic Technologies Inc. (Essex Junction, VT), prothrombin from Enzyme Research Laboratories (South Bend, IN), thrombin chromogenic substrate (H-D-Phe-Pip-Arg-pNA . 2HCl) from Molecular Innovations, Inc. (Novi MI), fluorogenic substrate I-1140 from Bachem Bioscience Inc. (King of Prussia, PA), Innovin from DADE Behring, Marburg, Germany, rabbit skeletal muscle myosin from Cytoskeleton, Inc. (Denver, CO) or Sigma, fatty acid-free and protease-free bovine serum albumin (BSA) from Sigma, trifluoperazine (TFP), (-)-blebbistatin, CK-1827452, and BTS from MP biochemical (Santa Ana, CA), Cayman Chemical Company (Ann Arbor, Michigan), Toronto Research Chemicals Inc (North York, Ontario, Canada), and Millipore Sigma (Burlington, MA), respectively. L-α-PS and L-α-PC (PC)(each from porcine brain) were from Avanti Polar Lipids (Alabaster, AL).

Peptide design and synthesis

3 peptides representing 129-161 amino acid residues of skeletal muscle myosin ELC and containing overlaps of 7-10 amino acids were synthesized using standard solid phase peptide synthesis and Fmoc chemistry by Synthetic Biomolecules (San Diego, CA) and purified to > 90% by HPLC (**Table 1S**).

Nineteen 18-40-mer peptides containing 6-22 amino acid-overlaps representing the skeletal muscle myosin neck region were synthesized using standard solid phase peptide synthesis and Fmoc chemistry by Cellmano Biotech Limited (Hefei, China), Shanghai Apeptide Co (Shanghai, China) or Shanghai Dechi Biosciences Co. (Shanghai, China), and purified to > 90% by HPLC. Peptide identity was confirmed by high resolution mass spectra (HRMS) that were recorded on an Agilent 1200 Series Accurate Mass Time-of-Flight (TOF) with an Aeris Widepore column (XB-C8, 3.6 μm particle size, 150 × 2.1 mm, flow: 0.5mL/min).

Table S1. Initial ELC Peptides screened for inhibition of myosin-enhanced prothrombin activation.

residues	*sequence*
ELC 129-144	**Ac-YEDFVEGLRVFDKEGN-NH2**
ELC 138-157	**Ac-RVFDKEGNGTVMGAELRHVL-NH2**
ELC 146-161	**Ac-GTVMGAELRHVLATLGEK-NH2**

Figure S1

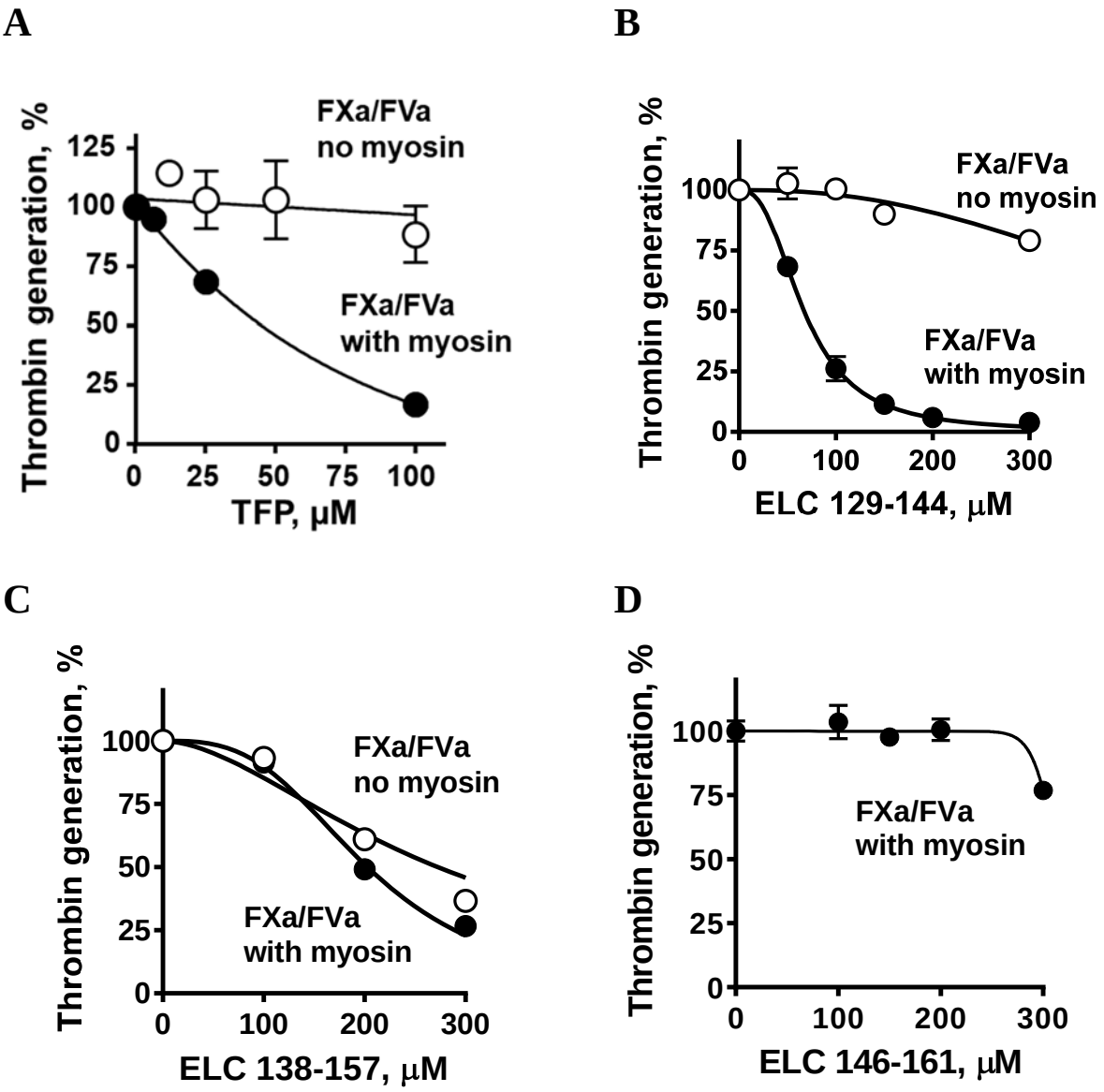

Figure S1. Inhibition of prothrombin activation by TFP or myosin peptides in the presence or absence of myosin. Varying concentrations of TFP (0–100 μM) (**A**) or peptides ELC129-144 (**B**), ELC138-157 (**C**) and ELC146-161 (**D**) (0–300 μM) were incubated with factor Va (5 nM, final) and factor Xa (0.2 nM, final) in TBSA plus 5 mM $CaCl_2$ at room temperature in the presence or absence of skeletal muscle myosin (2 nM, final). Thrombin generation was initiated by the addition of prothrombin (0.75 μM) in TBSA containing 5 mM $CaCl_2$. The reaction was quenched by adding EDTA (10 mM final) at 10 min. Thrombin formation was quantified by the rate of substrate (Pefa TH) hydrolysis. 100 % was the value for controls in the absence of added peptides. Each value represents the mean [± SEM] of at least triplicate determinations.

Figure S2

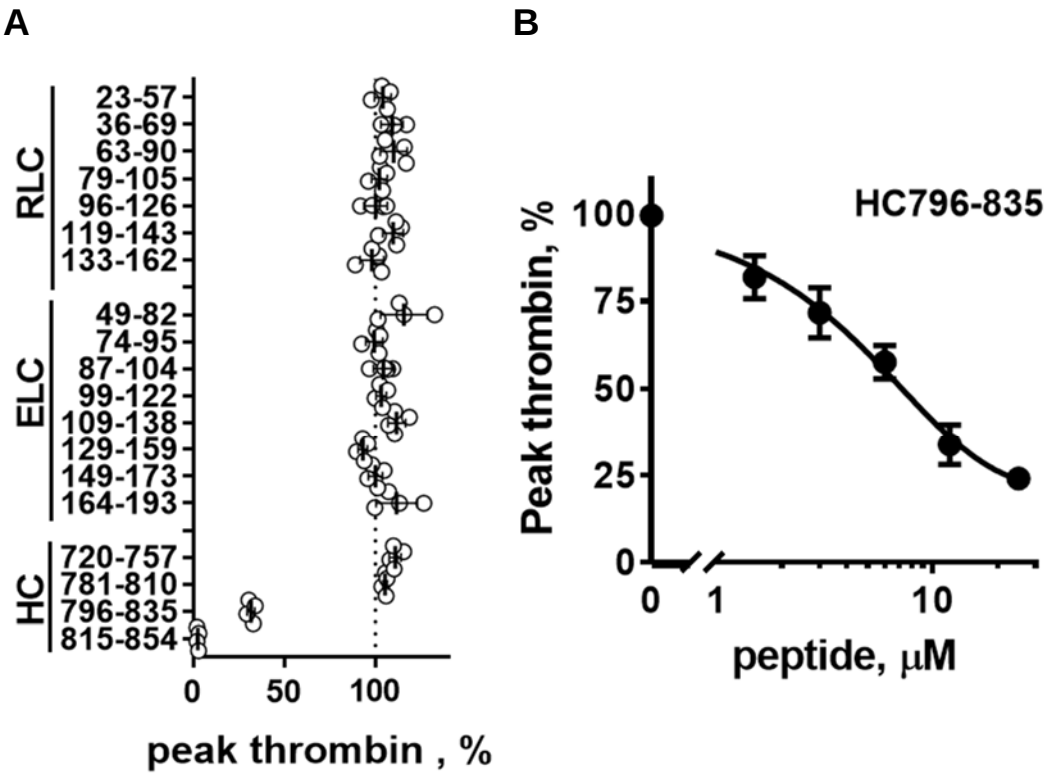

Figure S2. Peptide screening for their inhibition of Tissue Factor-induced thrombin generation in human plasma.
(**A**) 19 myosin-derived peptides (100 μM, final) were assayed for their ability to inhibit thrombin generation in human plasma, as described in Experimental procedures. 100 % was the peak thrombin value for controls in the absence of added peptides. Each value represents the mean [± SD] of quadruplicate determinations.
(**B**) Varying concentrations of peptide HC 796-835 (0–25 μM, final) were assayed for their ability to inhibit thrombin generation in human plasma, as described in **Experimental procedures.** 100 % was the peak thrombin value for controls in the absence of added peptides. Each value represents the mean [± SEM] of quadruplicate determinations.