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Fibrinolytic Activity of Cysteine Cathepsins and Role of Fibrin as a Reservoir to Sustain Proteolysis

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Cysteine cathepsins are powerful proteases first identified in lysosomes responsible for protein turnover, but now known to degrade collagen, elastin, and other matrix proteins. They are upregulated during atherosclerotic plaque development, and elevated cathepsin plasma levels are biomarkers for cardiovascular disease. The ability of cysteine cathepsins to hydrolyze fibrin and its role in hemostasis and resolution of fibrin based clots has not been well characterized, even though clotting cascades and fibrinogen conversion to fibrin can be initiated on the endothelial cell surface, and cathepsins are present in circulating blood where clots form. We hypothesize that cysteine cathepsins K, L, and S, key proteases implicated in cardiovascular disease, may have fibrinolytic activity and could play a role in fibrin clot degradation. To test this, fibrin gels were polymerized in 96 well plates by thrombin (1U/mL) cleavage of fibrinogen (2.5mg/mL). Polymerized fibrin gels were incubated for 24 hours with increasing amounts of recombinant cathepsins (cat) K, L, or S (0.15 – 1.5μg), or plasmin (0.75μg), the canonical fibrin degrading protease, to allow for proteolytic fibrin cleavage and solubilization of gels. Fibrin gels were imaged before and after degradation, and quantified by turbidity and densitometry to assess fibrinolytic activity. CatK, L, and S solubilized fibrin 48%, 38%, and 100%, respectively, with 1.5μg compared to plasmin solubilizing fibrin by 50% (Fig. 1A, n=3-5, $p<0.05$). Fibrin fragments released into supernatant due to proteolytic cleavage were collected and resolved with reducing SDS-PAGE to assess hydrolysis of α, β, and γ subunits of the fibrin molecule. There was a statistically significant loss of α and.or β fibrin polypeptide chains (Fig. 1B, n=4, $p<0.05$) with 1.5μg of catK, L, and S, compared to no enzyme control, indicating cathepsin fibrinolysis. Remnants of the fibrin gel were also collected and examined, which indicated similar subunit cleavage . Cathepsins inactivate and lose proteolytic activity over time with substrates, like elastin, with other substrates, like collagen, they are stabilized with extended lifetimes. To examine if fibrin was such a substrate, we tested samples with multiplex cathepsin zymography to identify active amounts of cathepsins. In the zymogram, active catL signal appeared at various molecular sizes that correlated with sizes of fibrin, fibrin fragments, and free catL alone (Fig. 1C), indicating its association with fibrin fragments and successive cleavage products. CatS only yielded active signal at the expected size, not bound to any fibrin fragments, and catK yielded no active signal. These studies demonstrate that cat K, L, and S are fibrinolytic, degrade α and β fibrin polypeptide chains, but produce different cleavage patterns than plasmin. CatL has a unique property of binding to fibrin and degradation products, retaining its activity. This suggests that catL adsorption to fibrin in gel or in solution can serve as a reservoir to sustain its activity for longer periods than expected. This may have potential implications for understanding cathepsins' role in clot resolution and will be the subject of further studies.