IDENTIFICATION OF PROTEASE(S) INVOLVED IN PARTIAL PROTEOLYSIS OF PLATELET DERIVED FV/Va

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Human coagulation factor V (FV) is an essential protein cofactor for the prothrombinase complex in the blood coagulation cascade. After being synthesized by liver hepatocytes, FV travels through the blood until being endocytosed and stored by megakaryocytes, platelet precursor cells. Multiple lines of evidence implicate this pool of FV as the physiologically relevant form in the clotting machinery. During the endocytic process FV is proteolyzed to a partially active form, however, the subcellular location and enzymes involved remain unknown. This project is aimed at investigating the location and identities of enzymes mediating this process. The problem was approached by both *in vitro* proteolysis assays and subcellular fractionation of platelets and CMK cells, a megakaryocyte like cell type. Furin and Cathepsin S were considered as candidate proteases based on their documented subcellular locations in the Golgi and endocytic pathway, respectively. During purified *in vitro* assays Furin did not cleave FV and Cathepsin S produced a cleavage pattern dissimilar to that found in platelet FV/Va. Plasma membrane permeabilization and fractionation of platelets into cytosolic and membrane fractions followed by *in vitro* proteolysis assays revealed that a cation-dependent cytosolic protease cleaves FV, but also that the actual protease(s) cleaving endocytosed FV are not in platelets. This is consistent with reported cleavage of FV by platelet calpain. To identify the location of protease(s) cleaving FV in the endocytic pathway subsequent to endocytosis, subcellular fractionation of CMK cells was performed using a continuous Iodixanol gradient. Results indicate clear separation of homogenized cellular material, and current work is utilizing *in vitro* FV proteolysis assays with subcellular fractions to identify the endocytic compartment where FV is proteolyzed subsequent to endocytosis. This will be followed up with liquid chromatography mass spectrometry to aid in identification of the protease(s) cleaving FV.