Functional assessment of Endo β-N-acetylglucosaminidase H-mediated deglycosylation of purified, plasma-derived factor V in vitro

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Cofactor, coagulation factor Va (FVa) is a key component of the prothrombinase complex which increases the rate of thrombin generation by approximately 10,000-fold. Thus, as the non-enzymatic cofactor of the protease factor Xa in the presence of calcium ions and activated platelets, they together allow for the generation of significant amounts of thrombin required for clot formation following injury. Factor V (FV), the precursor to FVa, is synthesized in the liver and circulates as two pools with ~25% stored in platelets and 75% flowing freely in an inactive state in plasma. Interestingly, we have observed functional and phenotypic differences between these pools of FV; the platelet pool is derived from endocytosis of the plasma pool and we speculate that post-translational modifications and packaging following endocytosis conspire to make the platelet FV the hemostatically-relevant pool. One notable modification is the disparity in high-mannose N-linked glycan on the light chain (Asn^{1982}) of factor Va. About 4-6 and 6-9 mannose residues are found in the platelet- and plasma-derived FV at Asn^{1982} respectively. In contrast, the numbers of high mannose glycans on the FVa heavy chain (Asn^{269}) are the same in both pools of FV suggesting a site-specific pruning of the Asn^{1982} glycan. We monitor in the present study the consequence of removing this glycan from the plasma-derived factor V on its functions in vitro and its ability to undergo endocytosis using megakaryocyte-like cell line, CMKs. Site specific removal of glycans at Asn-269 and 1982 were performed by incubating Factor V with Endo β-N-acetylglucosaminidase H. We observed that following treatment, there were no significant differences between inactivation of the FVa by APC mediated cleavage, and ability to clot in plasma based clotting assays when compared to the untreated samples. Lectin

binding assays, electrophoretic mobility shifts and mass spectrometry all confirm removal of some of the glycans. The catalytic efficiency of Endo-H treated FVa was only ~1.6-fold lower relative to the untreated control samples and both experimental samples were endocytosed by megakaryocytes. Whilst removal of the high mannose glycans appears to have little effect on the biochemical activities of factor Va in vitro, we speculate that the disparity in mannose residues might play a role during factor V trafficking *in vivo*.