Characterization of the megakaryocyte protease involved in the generation of the partially-activated, platelet-derived factor Va pool subsequent to its endocytosis from plasma

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ABSTRACT

Factor Va, the essential cofactor for thrombin generation, circulates in blood in two distinct pools. In contrast to plasma-derived factor V, which circulates as an inactive, single-chain procofactor, the platelet-derived molecule has undergone substantial proteolysis and is released from platelet α-granules at sites of vascular injury in a partially-active state. Although other physical differences, including a unique O-linked glycosylation on Ser^{692} of platelet-derived factor Va, have been noted between the two pools, their effects on cofactor function are unknown. As platelet-derived factor Va originates from endocytosis of the plasma procofactor by platelet precursors, megakaryocytes, we hypothesize that it is modified subsequently to form the unique platelet-derived cofactor. In the current study, the cleavage of ^{125}I-labeled plasma-derived factor V subsequent to its endocytosis by megakaryocytes was monitored by SDS-PAGE followed by western blotting analysis or autoradiography. Subsequent to factor V endocytosis, we observed a time-dependent increase in factor V proteolytic fragments in megakaryocyte lysates. Using various anti-factor V monoclonal antibodies, cleavage products similar in size to the factor Va light chain and heavy chain generated by activation of purified plasma-derived factor V with thrombin, as well as factor V activation intermediates, were identified, which is consistent with the formation of the active cofactor. Furthermore, megakaryocyte protease-derived factor V fragments were similar in size to those seen in platelets. These observations suggest that megakaryocytes express a proteolytic activity responsible for production of partially-activated, platelet-derived factor Va. Additional studies are necessary to identify the specific cleavage sites in endocytosed factor V, which will facilitate identification of the megakaryocyte protease(s). Future studies will also identify the cellular compartment(s) in which proteolysis occurs. These studies will contribute to a better understanding of how factor V is processed in megakaryocytes, and how this processing affects the activity of the platelet-derived factor V pool during hemostasis.