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FLUORESCENCE STUDIES OF THE BINDING OF FACTOR Va TO PHOSPHOLIPID VESICLES. S.Krishnaswamy and K.G.Mann, Dept. of Biochemistry, University of Vermont, Burlington, Vermont, U.S.A. 05405

The free sulphydryl groups in factor Va were analyzed under native and denaturing conditions using dithio-bis-nitrobenzoic acid (DTNB). The heavy chain (D) and the light chain (E) of factor Va were found to contain one free sulphydryl each under native conditions and following denaturation. Intact factor Va contained two free sulphydryls after denaturation while only one of these groups was accessible to DTNB under native conditions. Analysis of the rates of modification of the accessible sulphydryl in factor Va, and direct visualization of factor Va reacted with fluorescent sulphydryl modifiers and analyzed by SDS-PAGE, indicated that the accessible group was present in component D. Factor Va was labelled with the sulphydryl-directed flurophore N-pyrene-1-malemide with no loss in functional activity. Fluorescently modified Va (PYR-Va) contained between 0.83 and 0.91 moles pyrene and showed a concomittant loss in the free sulphydryls accessible to DTNB. Fluorescence polarization studies indicated that the binding of PYR-Va to phospholipid vessicles composed of phosphatidylcholine (PC) and phosphatidylserine (PS) was accompanied by a significant increase in the fluorescence polarization of the pyrene moiety. Systematic analysis of the binding of PYR-Va to PCPS (75%PC, 25%PS) indicated that the binding interaction was characterized by a $Kd=2.7x10^{-9}M$ and a stoichiometry of 42 monomeric phospholipids per lipid combining site. The binding of PYR-Va to PCPS was independent of added calcium ion and could be reversed by the addition of unlabelled factor Va. Analysis of the displacement curves indicated that native factor Va and PYR-Va mutually excluded each other with identical affinities ($Kd=2.5x10^{-9}M$). Isolated component D had no effect on the interaction between PYR-Va and PCPS, while isolated component E was capable of disrupting the PYR-Va-PCPS interaction ($Kd=3.1x10^{-9}M$) indicating that this subunit entirely mediates the interaction between Va and PCPS. No detectable binding of PYR-Va was observed when 100% PC vesicles were used and systematic variation of the PS content of the vesicles indicated that this parameter linearly influenced the stoichiometry of the PYR-Va-PCPS interaction with a minimal effect on the dissociation constant for the reaction. The data indicate that the factor Va-binding site is determined by the PS content of the lipid bilayer.
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INACTIVATION OF FACTOR Va BY PLASMIN
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The inactivation of Factor Va by plasmin was studied in the presence and absence of phospholipid vesicles and calcium ions. The action of plasmin resulted in a rapid loss of the ability of Factor Va to serve as a cofactor to Factor Xa , as judged by clotting assays and direct assays of prothrombin activation using the fluorophore, dansylarginine N-(3-ethyl-1,5-pentanediyl) amide (DAPA). The rate of Factor Va inactivation catalyzed by plasmin was markedly enhanced by the addition of phospholipid vesicles (PCPS), suggesting that the action of plasmin on Factor Va may be a membrane bound phenomena. Both Factor Xa and prothrombin were capable of protecting Factor Va from inactivation by plasmin. SDS-PAGE was utilized to correlate plasmin catalyzed proteolysis of Factor Va with the concomitant loss of activity. Data obtained with Factor Va and the isolated chains of the cofactor indicated that the light chain (E) was cleaved by plasmin to yield products similar to those obtained with Factor Xa and Activated Protein C (APC). The heavy chain (D) was found to be degraded by plasmin to produce proteolytic fragments distinct from those produced by Factor Xa and APC. The action of plasmin on single chain Factor V was notable for an initial, transient increase in total Factor V activity, followed by subsequent loss of activity, indicating the transient formation of active intermediates. SDS-PAGE analysis revealed the degradation of Factor V by plasmin to final, inactive products via several transient, higher molecular weight intermediates. These findings may be of some significance in pathophysiologic states in which systemic fibrinolysis may occur, possibly contributing to the depletion of clotting factors. The identification of such ongoing processes may eventually be facilitated by the observation that the degradation of Factor Va by plasmin leads to end products which may be unique to this interaction.
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FACTOR V IS ACTIVATED AND CLEAVED BY PLATELET CALPAIN: COMPARISON WITH THROMBIN PROTEOLYSIS.Robert W. Colman, Harlan Bradford, Anjanayaki Annamalai. Thrombosis Research Center, Temple University School of Medicine,Department of Medicine, Philadelphia, PA, USA

Platelets are known to process human and bovine factor V during secretion and/or membrane binding. We therefore studied the functional and structural changes produced in human factor V and Va by purified platelet calpain. A maximum increase in factor V coagulant activity of 2.5-fold over control incubations was observed for calpain (0.6 u/ml) at 25°C in comparison with a 10-fold increment for a thrombin (1 u/ml). Thrombin addition to reactions initiated by calpain resulted in further activation comparable to that of thrombin alone, while subsequent addition of calpain had no effect on the extent or pattern of the activation of factor V by thrombin. The cleavage pattern of factor V produced by these two enzymes are distinctly different. Calpain yields initial components of 210 kDa and 160 kDa within 1 min. Further digestion of the 210 kDa species give rise to polypeptides of 150, 140 and 120 kDa by 2 min with and increase in coagulant activity. The degradation of the 160 kDa polypeptide gives rise to smaller fragments of 130, 100, 90, and 87 kDa. Immunoblotting of these fragments with the monoclonal antibody B10 directed to factor V and the thrombin generated C1 fragments yields results demonstrating an immunological relationship to the calpain generated components of 210, 160, 140 and 120 kDa. Thus platelet calpain generates a complex cleavage pattern different from thrombin which may explain the partial activation observed.

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THE ROLE OF THE CARBOHYDRATE MOIETY OF HUMAN FACTOR V IN COAGULATION AND TURNOVER. T. Bruin, A. Sturk, J.W. ten Cate and M. Cath. Division of Haemostasis and Thrombosis, Academical Medical Centre, Amsterdam, the Netherlands.

The importance of the carbohydrate moiety of the human coagulation factor V molecule was investigated by its desialation and deglycosylation. Upon removal of 90% of the sialic acid residues a 1.5-2 fold increase in clotting activity was observed. Whereas up to 70% deglycosylation resulted in a parallel decrease in clotting activity. Thrombin induced activation of desialated factor V was unchanged, whereas deglycosylated factor V activation was impaired. The importance of the carbohydrate structure was further established by lectin incubation experiments. The distribution of carbohydrate in the thrombin induced activation fragments of factor V was investigated in lectin blot experiments with sialic acid-specific LFA, galactose-specific RCA-II and mannose-specific Con A. Carbohydrate residues were demonstrated in fragments B, C_1, D and F_1F_2. Interestingly, sialic acid was demonstrated in C_1, but galactose could not be shown. In fragment F_1F_2 ultimate galactose residues were found. The relevance of the carbohydrate moiety was further established by turnover experiments of native and desialated human factor V in rabbits. In contrast to the native factor V, desialated factor V was instantaneously cleared from the circulation.
In summary, these findings indicate an important role for the carbohydrate moiety in human coagulation factor V.