Tuesday, July 14, 1981

Oral Presentations

Coagulation V

Factors V and X

08:00–09:30 h

Coagulation VI

Factors V and X

09:45–11:00 h

Cinema 2

0259

08:00 h

A VARIANT OF FACTOR X THAT IS DEFECTIVE ONLY IN EXTRINSIC COAGULATION. R.M. Bertina, G.J.H. Alderkamp and E. de Nooy. Haemostasis and Thrombosis Research Unit, Leiden University Hospital, Leiden, The Netherlands.

Screening - prior to liver biopsy - of the blood of a 48-year-old patient with a negative anamnesis for bleeding complications, revealed a strongly prolonged prothrombin time (PT), while the APTT was normal. The PT prolongation was not the consequence of an antithromboplastin, vitamin K deficiency, oral anticoagulation or factor VII deficiency (FVII 0.5 U/ml). A more detailed analysis demonstrated the presence of an abnormal factor X molecule with the following properties: coagulation assays: 0.016 U/ml (thromboplastin), 0.43 U/ml (RVV/cephalin), 0.44 U/ml (kaolin/cephalin); spectrophotometric assay (RVV-X/S2337), 0.47 U/ml; immunological assay (Laurell), 0.50 U/ml. The low reactivity towards the extrinsic activator depends on the thromboplastin used: human brain, 0.016 U/ml; bovin brain, 0.11 U/ml.

The FX-variant has a normal electrophoretic mobility (crossed immunoelectrophoresis) and can be absorbed from plasma by $Al(OH)_3$. The variant has been purified from the patients plasma using affinity chromatography on anti-factor X Sepharose, followed by DEAE-Sephadex gradient elution.

The isolated variant shows the same low reactivity towards extrinsic activation. Comparison with isolated normal FX showed that it has the same molecular weight (NR 70,000; R 51,000 and 19,000). The heavy chain stains positive with the Schiff's reagent. Variant FX can be activated by RVV-X under formation of αXa (51,000), which in the presence of phospholipid is degraded to αβXa (48,000) and αγX (35,000).

Analysis of the kinetics of activation of normal and abnormal FX by FIXa, FIXa plus FVIII, or RVV-X revealed no essential differences. Only the activation by FVII-thromboplastin showed to be abnormal.

The complete absence of bleeding complications in this particular patient, might indicate that in vivo the extrinsic activation of factor X is only of minor importance.

0260

08:15 h

ISOLATION AND PROPERTIES OF INTERMEDIATES INVOLVED IN THROMBIN-CATALYZED CONVERSION OF BOVINE FACTOR V TO FACTOR Va. M.E. Nesheim and K.G. Mann, Hematology Research, Mayo Clinic/Mayo Foundation, Rochester, MN U.S.A.

Bovine Factor V was incubated briefly with bovine β-thrombin, causing partial conversion to Factor Va. QAE-cellulose chromatography in Ca^{++} of the partially activated material yielded 3 fractions. Electrophoresis in $DodSO_4$ indicated primarily starting material (Mr=330,000) in the first fraction; two peptides of Mr=150,000 and 200,000 in the second; and two peptides of Mr=200,000 and 90,000 in the third. The first of these latter fractions was active only after further incubation with thrombin, while the other was active without further thrombin catalyzed activation. After thrombin treatment both fractions had specific activities equal to that of fully activated, unfractionated Factor V. Incubation of either of these fractions with EDTA caused complete loss of activity and allowed isolation of the constituent polypeptides by further chromatography on QAE-cellulose in EDTA. The NH_2-termini of the 150,000 and 90,000 apparent Mr peptides had sequences identical to that of Factor V. The 200,000 apparent Mr peptides of the two fractions obtained by chromatography in Ca^{++} had NH_2-terminal sequences identical to each other but different from that of Factor V. The summed amino acid compositions of the Mr=200,000 and Mr=150,000 were equivalent to that of Factor V. Electrophoretic analysis indicated that thrombin converts the Mr=150,000 peptide to endproducts of Mr=90,000 and 71,000, and the Mr=200,000 peptide to endproducts of 83,000 and 31,000 apparent Mr. Recalcification of the individual isolated intermediates, followed by incubation with thrombin did not yield cofactor activity. Recombination of the recalcified intermediates followed by incubation with thrombin, however, generated samples with activities approaching that of unfractionated, activated Factor V. These data indicate that the intermediates of Mr=150,000 and Mr=200,000 arise by a single cleavage of Factor V, and that Factor Va comprises peptides derived from both of these intermediates.

0261

08:30 h

THE THROMBIN CATALYZED ACTIVATION OF HUMAN FACTOR V PROBED WITH A HYBRIDOMA ANTIBODY. J.A. Katzmann, M.E. Nesheim and K.G. Mann. Hematology Research, Mayo Clinic/Mayo Foundation, Rochester, MN U.S.A.

The peptides produced during the thrombin (II_a) catalyzed activation of human Factor V (HFV) were investigated using a murine monoclonal antibody. This antibody (HFV-1) binds HFV and activated HFV (HFV_a) and releases them at high ionic strength. HFV-1 coupled to sepharose was used to purify the single chain, 330,000 apparent MW procofactor molecule. Gel electrophoresis in SDS indicated that II_a-mediated activation proceeded with the appearance of intermediates with apparent MW of 270K, 205K and 150K and resulted in at least 3 end products with apparent MW of 110K, 93K and 70K. Aliquots of partially and fully activated HFV were chromatographed on the HFV-1 affinity resin. The peptides not associated with HFV_a were obtained by washing the column with 20 mM imidazole, 0.154 M NaCl, 5 mM $CaCl_2$, pH 6.5; the peptides noncovalently bound to the epitope-containing peptides were obtained by elution in the above buffer made 5 mM EDTA in place of Ca^{++}; and the epitope-containing peptides were eluted in buffer made 1.2 M in NaCl. The 150K intermediate was released in EDTA and the 205K and 270K peptides were eluted in high salt. Of the end products of activation, the 110K peptide was not retained by the column, the 93K peptide was released in EDTA, and the 70K peptide was released only at high ionic strength. These data indicate that the 270K and 205K intermediates, and the 70K end product share the antigenic site involved in the interaction with the monoclonal antibody. In addition a Ca^{++}-dependent interaction between the epitope-containing domain and the 150K intermediate and 93K end product is suggested. From this data and by analogy with the bovine protein, we suggest that HFV_a is a Ca^{++}-dependent multiple subunit protein consisting minimally of two peptides of apparent MW of 93,000 and 70,000.