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A NEW BLEEDING TENDENCY DUE TO HERITABLE HYPER α_2-MACROGLOBULINEMIA (α_2-MACROGLOBULIN-AKITA). Y. Endo, K. Iwamoto, S. Mamiya, H. Niitsu, T. Itoh and A. Miura. The Third Department of Internal Medicine, Akita University School of Medicine, Akita, Japan.

A heritable elevation in α_2-macroglobulin (α_2M) was identified in a 9-year-old girl with bleeding tendency and prolonged APTT to 49.1 sec (normal 27-38) and recalcification time to 438 sec (<180). Plasma α_2M level in her family tree i.e. sister, mother, maternal grand mother, father and paternal grand mother was 406, 380, 352, 339, 166 and 236 mg/dl (140-285), respectively. Thus it is thought to be an autosomal dominant disease, but other relatives show no apparent clinical syndrome. The significance of a possible causal association between elevated α_2M and prolonged APTT was showed.

Relationship between plasma concentration of α_2-macroglobulin and APTT

α_2-macroglobulin (mg/dl)	50	100	150	200	250	300	350
APTT (sec)	38.6	40.3	41.9	43.3	44.3	48.0	54.3

The activity of α_2M of the patient determined as trypsin-protein esterase was 351 mg/dl (197%), also. The α_2M of the patient showed normal mobility to anti-α_2M plasma with acceralated anodal mobility by crossed immunoelectrophoresis and normal mobility by immunofixation electrophoresis. In addition, by the analysis with SDS-polyacrylamidegelelectrophoresis we demonstrated no qualitative abnormality in α_2M of the patient. The new familial abnormality is tentatively designated "α_2-Macroglobulin -Akita".

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ACTIVATION OF HEPARIN COFACTOR II BY PHOSVITIN, A PHOSPHOGLYCOPROTEIN, AND OTHER PHOSPHATE-CONTAINING POLYANIONS. F.C. Church, R.E. Treanor, and H.C. Whinna. The Center for Thrombosis and Hemostasis, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 USA

We are characterizing the specificity of the polyanion-binding domain of the heparin/dermatan sulfate-dependent plasma protease inhibitor, heparin cofactor II (HCII). Various phosphate-containing polyanions accelerate the HCII-catalyzed inhibition of thrombin (T). Phosvitin, a phosphoprotein, enhances the HCII/T reaction at 25°C and pH 8.0 with the apparent second-order rate constant value (k_2) increasing from 5×10^4 M^{-1} min^{-1} (in the absence of phosvitin) to 8×10^7 M^{-1} min^{-1} as phosvitin increased from 0.05 to 30 ug/ml and then decreases as phosvitin is increased above 30 ug/ml. Apparent dissociation constant values for phosvitin-HCII and phosvitin-T are 450 nM and 10 nM, respectively. Polynucleotides accelerate the HCII/T reaction and have the following specificity (concentrations examined from 1-200 ug/ml): poly(guanylic acid) >> poly(adenylic acid, guanylic acid) > poly(inosinic acid) > poly(guanylic acid, uridylic acid) > poly(uridylic acid) = poly(adenylic acid) > poly(cytidylic acid). Polyphosphate anions (phosphate chain length, n, ranging from 5-100) enhance the HCII/T reaction. When compared at an equimolar phosphate concentration (1 mM), the rate was saturated at $n = 50$ with a maximum k_2 of about 5×10^7 M^{-1} min^{-1}. Ca^{2+} (or Mg^{2+})-phosvitin/polyphosphate anion complexes and salmon protamine-polynucleotide complexes have lost the ability to enhance the HCII/T reaction. Phosphopyridoxylated-HCII (lysine modified), with greatly reduced heparin cofactor activity, has lost its accelerating effect with phosvitin, polynucleotides and the polyphosphate anions. None of the above mentioned polyphosphate-containing compounds are effective at accelerating either the HCII-catalyzed inhibition of chymotrypsin or the antithrombin III-catalyzed T reaction. Our results suggest that (i) HCII is activated by the multiple negative charges of phosphate polyanions but they alone are not sufficient; (ii) the effective phosphate polyanions must also possess a specific conformation for maximum activity; and (iii) the phosphoserine-containing protein, phosvitin, can serve as a "template" for HCII/T.

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INACTIVATION OF HEPARIN COFACTOR II (HC II) BY POLYMORPHONUCLEAR LEUKOCYTES (PMNL). P. Sié, D. Dupouy, F. Dol and B. Boneu. Laboratoire d'Hémostase, Centre de Transfusion Sanguine, 31052 Toulouse, FRANCE.

Several authors have shown that antithrombin III (AT III) was catalytically inactivated by neutrophil elastase, an observation relevant to pathophysiological processes in the vicinity of inflammatory sites. The aim of this study was to investigate whether HC II, another natural thrombin inhibitor, is also inactivated by PMNL.

A rapid loss of HC II activity occured upon incubation with fresh human PMNL stimulated by phorbol myristate acetate (T1/2 1 uM HC II, 0.35 10^8 cells/mm^3 : ~2 min) or with PMNL extracts prepared by nitrogen cavitation. Antithrombin (dermatan sulfate cofactor) and antichymotrypsin activities of HC II were lost at the same rate. Resting PMNL were ineffective. Inactivation was prevented by several serine-protease inhibitors but was Ca^{++}/Mg^{++} independent. Inactivation coincided with the formation of a 54 KD peptide after a first non-inactivating degradation into a 62 KD peptide (native HC II : 76 KD). These reaction products are reminiscent of those described upon incubation with proteinase I from Echis carinatus venom. HC II was inactivated more rapidly than AT III (T 1/2 of AT III in the same conditions ~15 min). However, heparin (1-10 ug/ml) strongly accelerated the rate of AT III inactivation and slightly protected HC II, thus reversing the order of inactivation. Dermatan sulfate had no effect on this process.

In conclusion, this study shows : 1)both AT III and HC II are rapidly inactivated by PMNL enzymes, thus favoring locally thrombin-mediated processes ; 2) heparin increases AT III degradation by PMNL, a possible route of catabolism operating in patients with low AT III levels during heparin treatment.

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MOLECULAR HETEROGENEITY IN FAMILIAL HEPARIN COFACTOR II DEFICIENCY. T.R.Andersson,M.L.Larsen and U.Abildgaard. Haematological Research Laboratory,Aker Hospital,Oslo 5 Norway.

In our normal material of 379 blood donors,3 individuals had values below mean -2.5 SD (below 56%).Further studies revealed hereditary deficiency in two of these individuals. In the family study,5 out of 7 individuals had heparin cofactor II (HC II) values below 56%. In only one of these 5 a history of DVT was obtained. In deceased members of her family,however,frequent episodes of thromboembolic disease had occurred.
Crossed immunoelectrophoresis (CIE) was performed in plasma from deficiency individuals from both families. Heparin in the first dimension gave a pattern similar to that observed with normal pooled plasma. The inactivation of thrombin by HC II is preferentially accelerated by dermatan sulfate (DS).HC II consumption in in vitro coagulation is increased by DS rather than by heparin. This prompted the addition of DS to the first dimension,which makes the antigen move faster, but produced no alteration in the antigenic pattern or size, neither in pooled plasma nor in a family member with normal activity. In family members with low HC II activity,DS in the first dimension,resulted in an abnormal CIE pattern with two distinct precipitation arcs with identity pattern. Molecular heterogeneity of HC II has previously not been reported.

Screening 70 individuals who had sustained thrombosis before the age of 50,no values suggesting congenital deficiency were encountered.

Conclusion. The CIE findings suggest that plasma of affected family members contain two types of HC II molecules of immunological identity. One type shows the normal accelerated mobility in the presence of DS and the other lacks this effect. The abnormal electrophoretic pattern becomes,however,first apparent when DS is added to the first dimension. When looking for molecular defects in congenital HC II deficiency,it is important that the first dimension is run with DS.