

A FAMILY OF HEREDITARY DYSPLASMINOGENEMIA.

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The proband was 41 year-old male without any history of thrombosis. Recurrent thrombosis were developed after he had received an accidental contusion on his chest, and coagulation analysis revealed no abnormality except for low plasminogen activity, about 20% of normal value.

No thrombotic trait was claimed by patient's family but there was consanguineous marriage in his parents. Results of coagulation analysis of 15 members of this family were as follows; platelet count, platelet aggregation and clot retraction were normal. Activity of all coagulation factors and antigenicity as well as activity of α_2 -M, α_2 -PI, AT-III and α_1 -antitrypsin were within normal ranges. Plasminogen activity, however, were 20-60% of normal value in 9 out of 15, it was 80-120% in 6.

Plasminogen of each member was isolated by affinity chromatography with lysine-Sepharose column and kinetic study was performed with these isolated plasminogen. Km's of all members were ranged in 170-230 μ M/L as compared to 170 μ M/L in normal control. But Vmax's were distributed from 0.19 to 3.28/min.ml as compared to 3.1 in normal control. On the other hand, antigenicity of plasminogen of these members were ranged in 10-14.2 mg/100ml except for a case with 17.6 mg/100ml.

SDS-gel electrophoresis revealed that activation of this plasminogen with SK yielded two subunits, H and L, and plasmin formed a complex with α_2 -M, similar as normal plasmin did.

It was concluded that this family had dysplasminogenemia inherited by a dominant mode, and plasminogen of these patients was a heterologous mixture composed of normal and abnormal plasminogen.

IMMUNOLOGICAL DETERMINED PLASMINOGEN GROUPS AND ITS

POSSIBLE BIOLOGICAL RELEVANCE. V. Sachs, R. Dörner and E. Szirmai. Department of Immunohematology and Blood Transfusion of the University of Kiel and Department of Nuclear Hematology and Radiation Biology I.N.E. and Associated Universities, Stuttgart, F.R.G.

Anti human plasminogen sera of the rabbit precipitate human plasma in the agar gel diffusion test by means of intra-basin absorption with plasminogenfree human plasma with three different types: type I is represented by one strong precipitation line, type II by two lines, a big one and a small one, and type III by three slight but distinct lines. The following frequencies of the different types have been observed in a sample of 516 human plasmas: type I 65%, type II 33% and type III 2%. Suppose the types are phenotypical groups of a diallelic system where the types I and III represent the homozygous genotypes and the type II the heterozygous the estimated gene frequencies are in good agreement with the expected values. There is also a good agreement of the distribution of plasminogen groups determined by electrofocussing from RAUM et al. and HOBART. The plasminogen groups possibly may have also a biological meaning because the plasmas of type III always have a lesser fibrinolytic activity than the plasmas of the other types.

NEW SYNTHETIC PEPTIDE SUBSTRATES OF -VAL-pNA TYPE FOR AN INSOLUBLE FIBRINOLYTIC SERINE ENZYME EXTRACTED FROM HUMAN SPLEEN. U. Okamoto, Y. Nagamatsu, N. Horie, Y. Okada and Y. Tsuda. Faculty of Nutrition, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Kobe 673, Japan.

An insoluble spleen fibrinolytic serine proteinase (SFP) extracted from the human spleen with 2 M NaClO₄ and partially purified by the authors was characterized with its enzymatic capability to split fibrin and fibrinogen at neutral pH. Its substrate specificity was much limited; any synthetic peptide substrates for factors of coagulation and fibrinolysis were not hydrolyzed. The present studies were therefore made to search for SFP peptide substrate which mimicked possible natural substrates, using the partially purified enzyme preparation showing single protein band possessing fibrinolytic activity on polyacrylamide gel disk electrophoresis.

Despite of fact that various chromogenic peptide substrates having principal structure of -Arg-pNA and -Lys-pNA and various esters of Lys, Arg, Try, Phe and Tyr were not decomposed by SFP, a clue was however found that Suc-Ala-Ala-pNA (substrate for elastase) was decomposed by SFP but very slowly. Then, trial to mimic a part of insulin B chain (a substrate for elastase) led us to reach Suc-Tyr-Leu-Val-pNA which was well amidolyzed by SFP with K_m of 0.17 mM and K_{cat}/K_m (M) of 22,600. Further, Suc-Ala-Tyr-Leu-Val-pNA was found more appropriate substrate than the former. It was noteworthy that ratio of SFP susceptibility of Suc-Ala-Pro-Val-pNA, Suc-Ala-Pro-Ala-pNA and Suc-Ala-Pro-Leu-pNA was approximately 9:1:0.

Results obtained so far concluded that synthetic peptide substrates appropriate for SFP are dominantly characterized by the C-terminal-Val; other part amino acid sequences is also significant however.

INTRINSIC FLUORESCENCE CHANGES AND EXPOSURE OF SULFHYDRYL GROUPS ON α_2 -MACROGLOBULIN DURING REACTION WITH SERINE PROTEASES. D.L. Straight and P.A. McKee. Howard Hughes Med. Inst., Duke Univ. Med. Ctr., Durham NC, USA.

The reactions of serine proteases, methyl amine (CH₃NH₂) and denaturants with α_2 -macroglobulin (α_2 M) were studied by intrinsic fluorescence, SDS gel electrophoresis and sulfhydryl (SH) group titration. The cleavage of α_2 M by trypsin, thrombin and elastase, as followed on SDS gels after reduction, corresponded to an increase in protein fluorescence. Both proteolysis and fluorescence were maximal at two moles of each enzyme per mole of α_2 M. The second order rate constant for inhibition of thrombin's fibrinogen clotting activity by α_2 M was the same as the rate constant for fluorescence change during reaction of α_2 M with thrombin. Amines (e.g., CH₃NH₂) that inactivate α_2 M caused an increase in protein fluorescence that correlated with the rate of loss of α_2 M's ability to protect trypsin from soybean trypsin inhibitor. Prior treatment of α_2 M with proteases or amines abolished the ability of either to enhance fluorescence. We found that CH₃NH₂ also caused exposure of \sim 4 SH groups per molecule of α_2 M when titrated with 2,2'-dipyridyl disulfide or 5,5'-dithiobis (2-nitrobenzoic acid). The rate of SH group exposure by CH₃NH₂ corresponded to the rate of protein fluorescence change induced by CH₃NH₂. The reaction of α_2 M with proteases gave similar results. Denaturation of α_2 M in 6 M guanidine.HCl (GnHCl) at neutral pH also exposed SH groups, but denaturation in 6 M GnHCl at acid pH, where the stoichiometric reaction of amines with α_2 M occurs very slowly, did not expose SH groups. This indicates that a conformational change is not solely responsible for this phenomenon. We conclude: 1) the stoichiometric reactions of proteases and amines with α_2 M coincide with a measurable increase in protein fluorescence; 2) two moles of trypsin, thrombin or elastase bind to one mole of α_2 M; and 3) these reactions result in exposure of \sim 4 SH groups per α_2 M molecule (probably one per subunit) by cleavage of non-peptide bonds which are readily susceptible to nucleophilic attack.