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CHARACTERIZATION OF THE GENE FOR HUMAN HISTIDINE-RICH GLYCOPROTEIN. T. Koide. Dept. of Biochemistry, Niigata University School of Medicine, Niigata 951, Japan and Dept. of Biochemistry, University of Washington, Seattle, WA 98195, USA.

Human histidine-rich glycoprotein (HRG) is a single-chain glycoprotein in plasma which is considered to modulate a coagulation and fibrinolysis system with the ability to bind to heparin, plasminogen, fibrinogen, thrombospondin, etc. Recently we have elucidated the primary structure of HRG by determining the nucleotide sequence of its cDNA, and showed that HRG is composed of several different types of internal repeats, each one of which shows considerable homology with the functional and/or structural domains of other proteins including high molecular weight kininogen, antithrombin III, cystatins, and proline-rich protein and peptide. Thus, the multifunctional property of HRG was suggested to be due to its multi-domain structure. In the present studies, a human genomic DNA library, cloned in the bacteriophage vector Charon 4A, was screened for HRG gene using a full-length cDNA coding for human HRG as a probe. A total of 7 clones were isolated from 6 x 10^5 phage and each was plaque purified. The entire HRG gene is represented in 3 genomic inserts with overlapping sequences that carry human DNA spanning 30 kb. Overlapping gene fragments were subcloned into pUC9 and characterized by Southern blot hybridization using 5' and 3' end probes isolated from human HRG cDNA and by DNA sequencing. These studies have shown that the gene for human HRG spans about 9 kb and consists of at least 5 exons and 4 introns. The putative histidine-rich region consisted of 12 tandemly repeated sequences of a 5 amino acid segment and 2 proline-rich regions contiguous to it are likely to be involved within one exon.

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MODIFICATION OF GLUTAMIC AND ASPARTIC ACID RESIDUES OF PLASMINOGEN INHIBITS ITS ABILITY TO FORM AN ACTIVE PLASMINOGEN-STREPTOKINASE COMPLEX. D.S. Holloway (1,2), L. Summaria (1), R.C. Wohl (3), and J.A. Caprini (1,2). (1) Evanston Hospital, Evanston, IL, (2) Northwestern University Medical School, Chicago, IL, and (3) University of Chicago, Chicago, IL, USA.

Plasminogen binds to streptokinase in a 1:1 molar complex that has activity as a plasminogen activator. This function of plasminogen, as a cofactor for streptokinase conversion of plasminogen to plasmin, was studied after treatment of Glu-, Lys-, and Mini-plasminogens with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). Amino acid analysis showed that both aspartic and glutamic acid residues were modified by EDC. Activity of the complex formed between streptokinase and the modified plasminogen was measured using the chromogenic substrate H-D-Val-Leu-Lys-pNA. Plasminogen, 2.8 uM, was incubated with 40 mM EDC in 50 mM MES buffer, pH 6.0, at 25°C. At various times while reacting plasminogen with the EDC, aliquots were removed for assay. Plasminogen function was assayed by mixing with a slight molar excess of streptokinase for 1 min at 37°C, followed by reaction with 0.1 M substrate, and absorbancy monitored at 405 nm. Modifications of 20% of the glutamic and aspartic acid residues occurred after treatment of plasminogen with EDC. This resulted in 80 to 90% inhibition of activation in all three types of plasminogen. Glu- and Lys-plasminogens reacted more quickly with the EDC than did Mini-plasminogen, with 50% inhibition occurring after 16 ± 5, 16 ± 4, and 67 ± 13 min reaction time with EDC for Glu-, Lys-, and Mini-plasminogens, respectively. Maximum inhibition of activation occurred within 1 hr reaction with EDC for Glu- and Lys-plasminogens but required 2.5 hr for Mini-plasminogen. The time courses for activation inhibition and the modification of the glutamic and aspartic acids of treated Mini-plasminogen were compared. A significant decrease in activation occurred (52%) concomitant with modification of only one or two glutamic acids, followed on further reaction with EDC by more loss of activatability as more glutamic and aspartic acids were modified. The inability of plasminogen to form an active plasminogen-streptokinase complex after modification with EDC indicates that glutamic and aspartic acid residues are involved in the binding site of plasminogen for streptokinase.

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THE CONTRIBUTION OF ENDOGENOUS UROKINASE (UK) AND TISSUE PLASMINOGEN ACTIVATOR (t-PA) TO SPONTANEOUS CLOT LYSIS IN PLASMA. V. Gurewich, F. Emmons and R. Pannell. Vascular Laboratory, St. Elizabeth's Hospital, Department of Biomedical Research, Boston, MA, USA.

Spontaneous lysis of ^{125}I-labeled clots was measured in order to study the relative contributions of pro-UK, t-PA and contact activation. Clots were made up from 0.3 ml plasma, platelet rich plasma (PRP) or 2% human fibrinogen (Kabi) and suspended in plasma (3 ml) prepared from blood freshly collected onto citrate. The clots were preincubated to inactivate any residual thrombin which could inactivate pro-UK. Antibiotics were added to suppress bacterial growth. Lysis was approximately linear and went to completion with plasma clots in 12-16 d. and with fibrinogen clots in 6-10 d. Spontaneous lysis was inhibited by aprotinin at >200 KIU/ml and by antibody to t-PA. When the latter was added at t 2-5 d., clot lysis was arrested at each time point suggesting that t-PA was predominantly responsible for the lysis and not just for its initiation. Addition of antibody to UK or immunodepletion of plasma had little effect on spontaneous lysis of plasma or of fibrinogen clots, but retarded lysis of PRP clots. Only after ≥20 ng/ml of pro-UK were added to plasma, was a dose-responsive acceleration of clot lysis observed. The addition of antibodies to t-PA inhibited clot lysis by the added pro-UK (20-80 ng/ml) and greatly prolonged the lag phase of clot lysis by higher concentrations of pro-UK (100-200 ng/ml). Contact activation by dextran sulfate (1-5 μM) had no effect on spontaneous clot lysis but potentiated the effect of ≥ 20 ng/ml added pro-UK. It was concluded that 1) The UK content of resting plasma (3-5 ng/ml) contributes little to lysis of platelet-free clots. 2) The presence of physiological concentrations of t-PA is essential for lysis by endogenous UK and markedly potentiates fibrinolysis by added pro-UK (20-200 ng/ml). 3) For endogenous UK to contribute to fibrinolysis, some amplification of its effect by a cofactor other than t-PA seems to be required. The results indicate augmentation of endogenous pro-UK induced fibrinolysis by platelets and that contact activation stimulates clot lysis by added pro-UK.

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CHARACTERIZATION OF RECOMBINANT HUMAN SINGLE-CHAIN LOW MOLECULAR WEIGHT UROKINASE (RE-SC-LUK). W.A. Günzler, B. Wolf and L. Flohé. Grünenthal GmbH, Center of Research, D-5100 Aachen, FRG.

RE-SC-LUK obtained from recombinant E. coli bacteria showed a molecular mass similar to that of recombinant two-chain LUK (RE-TC-LUK) as judged from SDS-PAGE. By "Western" blot analysis immunoreactivity of RE-SC-LUK was observed with monoclonal antibodies directed against the B chain but not with those against the A chain of urokinase. N-terminal sequence analysis of RE-SC-LUK showed identity to the A_1 chain of RE-TC-LUK and provided evidence for its single-chain nature, i.e. integrity of the Lys-Ile bond which is split in TC-UK. In all other respects structural identity of RE-SC-LUK and RE-TC-LUK was demonstrated by fingerprinting of fragments. Similar to recombinant pro-urokinase (RE-SCU-PA), RE-SC-LUK exhibits only marginal amidolytic activity, which is greatly enhanced by treatment with plasmin, but considerable fibrinolytic activity in a fibrin agar plate test.

Thus, RE-SC-LUK is characterized as a fragment (residues 136 - 411) of RE-SCU-PA, which lacks the "growth factor" and "kringle" domains. Moreover further evidence is provided that a free N-terminus of the B chain is essential for amidolytic but not for fibrinolytic activity of urokinase in more complex systems.