0255

10:00 h

ISOLATION AND CHARACTERIZATION OF A cDNA CLONE CODING FOR α_1-ANTITRYPSIN. K. Kurachi, T. Chandra, S. L. C. Woo and E. W. Davie. Dept. of Biochemistry, Univ. of Washington, Seattle, WA 98195, and Howard Hughes Medical Institute Laboratory, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030, U.S.A.

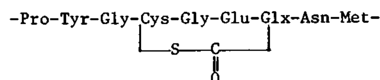
Poly(A)-RNA enriched for α_1-antitrypsin was isolated by specific immunoprecipitation of baboon liver polysomes. Alpha$_1$-antitrypsin consisted of greater than 90% of the cell-free translation products of this mRNA. A double-stranded cDNA was synthesized by using reverse transcriptase and made blunt-ended with nuclease S1. After tailing with dCTP and terminal transferase, the double-stranded cDNA was annealed to pBR322 DNA. The latter DNA had been cleaved previously at the single Pst I site and similarly tailed with dGTP. The resulting plasmids were used to transform Escherichia coli strain RR1. Clones that hybridized to ^{32}P-labeled cDNA synthesized from the α_1-antitrypsin-enriched mRNA were then identified. The recombinants containing baboon cDNA inserts were further screened by a solution hybridization assay with [^3H]cDNA synthesized from the enriched mRNA. The cDNA inserts from the positive clones were then sequenced to identify clones containing α_1-antitrypsin. One insert, designated pBaαA1, was found to code for the carboxyl-terminal region of α_1-antitrypsin. It also contained a noncoding region of 76 base pairs and a poly(A) tail of 60 base pairs.

0256

10:15 h

PRIMARY STRUCTURE OF α_2-MACROGLOBULIN. L. Sottrup-Jensen, T.M. Stepanik, P. Lønblad, D.M. Rider, T.E. Petersen and S. Magnusson. Department of Molecular Biology, University of Aarhus, Aarhus, Denmark.

The α_2M tetramer of M_r 725,000 consists of four apparently identical single polypeptide chain monomers of M_r 180,000, corresponding to about 1450 amino acid residues. At the present stage of our sequence determination 1383 residues can be accounted for in three segments of continuous sequence namely the N-terminal (residues 1-440); the "middle" of 298; the C-terminal of approx. 645 residues. The eight oligosaccharide prosthetic groups are glucosamine-based and attached to Asn-residues, five in the N-terminal, three in the C-terminal segment. 25 CNBr-fragments have been found, 22-Met-residues have been overlapped. 23 Cys-sequences have been determined so far and ten -S-S-bridges identified. The Cys-residue (approx. no. 472 from the C-terminal in each chain) of the sequence



is thiol-ester linked to the γ-carboxyl of the Glx-residue in native α_2M which has not been reacted with proteinase or "inactivated" with methylamine or by heat inactivation.
Neither extensive internal sequence homology, nor homology with other proteinase inhibitors of known primary structure has yet been observed.

0257

10:30 h

MECHANISM OF PROTEINASE-BINDING TO α_2-MACROGLOBULIN. L. Sottrup-Jensen, H.F. Hansen, S.B. Mortensen, T.E. Petersen, S. Magnusson and H. Jörnvall*. Department of Molecular Biology, University of Aarhus, Aarhus, Denmark and *Department of Chemistry, Karolinska Institute, Stockholm, Sweden.

Peptide-bond cleavage in the bait-region of the α_2M polypeptide chains, associated with proteinase-α_2M complex formation was shown to occur in the sequence: GLRVGFYESDVMGRG HARLVH (part of the 298-residue "middle" segment) such that plasmin, thrombin and trypsin cleave the Arg-Leu (RL) bond, elastase mainly the Val-Gly (VG) and partly the Gly-Phe (GF) bonds. Thus, in the first step of complex formation the proteinase active site binds to the bait region of α_2M.
J.B. Howard found that heat inactivation of α_2M causes cleavage of a Glu-Glx bond, and inactivation with CH_3NH_2 leads to incorporation of CH_3NH_2 on the γ-carboxyl of the same Glx-residue. We have found that reaction of α_2M with trypsin or elastase but not trypsinogen causes the 2:1 stoichiometric appearance of up to 4 SH-/tetrameric α_2M for up to 2 trypsin/2 elastase bound. The -SH was found to be the Cys-SH of thiol-ester-containing PYGCGEZNM sequence. Inactivation of native α_2M (by CH_3NH_2, heating, dissolving in 2 M guanidine or 1.6% SDS at 50°C) also led to the appearance of up to 4 SH for 4 CH_3NH_2 incorporated per tetrameric α_2M. Competition between trypsin and CH_3NH_2 for the thiol-ester site proved that trypsin binds to the same Glx-γ-carboxyl that incorporates CH_3NH_2. Thus, the second step of complex formation involves the thiol-ester site γ-carboxyl of α_2M and a proteinase-site other than its active-site (since the complex is active against small substrates). Other compounds with nucleophiles, e.g. putrescine or insulin, when added with trypsin, were also incorporated into α_2M. The thiol-ester mechanism may be associated with the rapid elimination of proteinase α_2M-complexes, particularly since the recent finding by B.F. Tack that complement factor C3 has an identical thiol-ester-containing sequence, points to at least one common function of these proteins.

0258

10:45 h

EFFECT OF HEPARIN AND PROTAMINE ON THE ANTITHROMBIN ACTIVITY OF α_2-MACROGLOBULIN. J.N. Shanberge, J. Love and N. Swanborg. Hemostasis and Thrombosis Research Laboratory, Department of Clinical Pathology, William Beaumont Hospital, Royal Oak, Michigan, USA.

It has been shown that certain fractions of porcine mucosal heparin may form complexes with α_2-macroglobulin but still retain their capacity to activate antithrombin III. These heparin fractions are not neutralized by protamine. The effect of heparin and protamine on the antithrombin activity of α_2-macroglobulin has now been studied.
Plasma to which a tritium-labelled heparin was added was chromatographed on Sephadex G-200. The macromolecular fractions, in which there is heparin (indicated by radioactivity) and α_2-macroglobulin (demonstrated by radial immunodiffusion), have no immediate inhibitory action on the thrombin clotting time (TCT) of fibrinogen but do inhibit the TCT of whole plasma. In addition, these fractions have progressive antithrombin activity (PATA) but less than that in fractions containing α_2-macroglobulin obtained with non-heparinized plasma. Fischer et al stated that heparin complexes with thrombin to prevent its inactivation by α_2-macroglobulin. However, when the heparinized plasma is "neutralized" with protamine sulfate prior to chromatography, the PATA of the macromolecular fractions is reduced further even through these fractions still have immediate inhibitory action on the TCT of plasma. When the same concentration of protamine is added to non-heparinized plasma, the PATA in the macromolecular fractions is not altered. Treatment of the macromolecular fractions from heparinized plasma with triethylaminoethyl cellulose removes the immediate inhibitory action on the TCT of plasma but does not affect the PATA of the fractions. How the heparin and protamine interact to decrease the PATA of α_2-macroglobulin containing fractions is not as yet determined. Recently it was found that a similar complexing of heparin fractions with α_1-antitrypsin may occur in the same way as with α_2-macroglobulin.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.