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POSTER SYMPOSIUM XVIII

Thrombosis: Structure and Activities of Antithrombin III.

ISOLATION OF HUMAN ANTITHROMBIN-III BY AFFINITY CHROMATOGRAPHY ON HEPARIN-AGAROSE. W.H. Holleman, L.J. Coen, J.O. Capobianco, G.H. Barlow, Dept. of Biochemistry, Abbott Laboratories, North Chicago, Illinois 60064.

Methods were developed for the isolation of gm. quantities of human antithrombin-III (AT-III) from Cohn Fraction IV-1 of human plasma using heparin covalently attached to agarose. Attachment of heparin carboxyl groups to alkylamino-agarose yielded a support with no affinity for AT-III. Linkage via the heparin hydroxyl groups yielded a support with approximately 1 mg of heparin/ml agarose and with a low capacity for binding AT-III. Linkage of heparin to agarose thru its amino groups yielded a heparin-agarose with the highest capacity for AT-III. Reaction of heparin containing a free α-amino group with cyanogen bromide activated agarose resulted in agarose substituted with 5 mg of heparin/ml. The conditions of buffer, pH, ionic strength and temperature which maximized AT-III binding were 0.05M sodium phosphate, 0.02M sodium citrate, 0.15M NaCl, pH 8.3 and 4°. The heparin-agarose bound 0.1-0.2 mg of AT-III/ml. The AT-III isolated by affinity chromatography was further purified by gel permeation and yielded a homogeneous product as judged by polyacrylamide disc gel electrophoresis of native and reduced protein and by sedimentation velocity ($S_{20,w}$ = 4.1). This material had an activity of 1700 units/A_{280} as measured by inhibition of human thrombin. The AT-III is stable to heating at 60° for 10 hours in a buffer of 0.5M sodium citrate at pH 7-8. Injection of bovine thrombin (3000 units/Kg) into heparinized dogs (150 units/Kg) decreased circulating AT-III levels to 50%.
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THE PRIMARY STRUCTURE OF ANTITHROMBIN-III. T. E. Petersen, G. Dudek-Wojciechowska, L. Sottrup-Jensen and S. Magnusson. Department of Molecular Biology, University of Aarhus, Denmark.

Human antithrombin-III is a single-chain glycoprotein with three disulfide bridges and four prosthetic glucosamine-based oligosaccharide groups. The disulfide bridges have been established. In four fragments of 208, 168, 3 and 46 amino acid residues, resp. 415 of the appr. 425 residues have been sequenced. The four oligosaccharide groups are attached to four Asn-residues within a sequence region of 95 residues. No extensive sequence homology with the trypsin inhibitors has been observed. One chymotryptic peptide was found to be a substrate for bovine factor X_a, cleaving the arginyl bond in the sequence -Ile-Val-Ala-Glu-Gly-Arg-Asp-. A second peptide is cleaved by thrombin. It is not clear whether these sites are inhibitor sites in the native molecule. Other possible candidates for inhibitor sites are a -Val-Leu-Ile-Leu-Pro-Lys-Pro- sequence (similar to the sequence 40-48 of hirudin, which also includes a -Pro-Lys-Pro- sequence) and also the C-terminal sequence -Gly-Arg-Val-Ala-Asn-Pro-Cys-Val-Lys.