0899

THE ACTIVE SITE OF ANTITHROMBIN. RELEASE OF THE SAME PROTEOLYTICALLY CLEAVED FORM OF THE INHIBITOR FROM COMPLEXES WITH FACTOR IX_a, FACTOR X_a AND THROMBIN. I. Björk, C.M. Jackson, H. Jörnvall, K.K. Lavine, K. Nordling and W.J. Salsgiver. Department of Medical and Physiological Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO, USA and Department of Chemistry, Karolinska Institutet, Stockholm, Sweden.

Reactions between near-equimolar amounts of antithrombin and Factors IX_a or X_a resulted in the formation of a free, proteolytically modified, two-chain form of the inhibitor, in addition to the inactive antithrombin-protease complexes. The modified antithrombin produced by either enzyme behaved electrophoretically identical to the previously characterized modified inhibitor formed in the reaction with thrombin. Like in the latter reaction, the formation of the modified antithrombin by Factor X_a was increased in the presence of heparin, while only small amounts were produced by Factor IX_a both in the absence and presence of the polysaccharide. Amino-terminal sequence analyses of the isolated modified inhibitor formed by Factor X_a showed that a single Arg-Ser bond in the carboxy-terminal end of the inhibitor had been cleaved. This cleavage site is identical to that previously identified in free thrombin-modified antithrombin. The antithrombin-Factor IX_a and antithrombin-Factor X_a complexes were purified and dissociated by ammonia or hydroxylamine. The dissociation products of both complexes were free enzyme and a modified two-chain form of the inhibitor. Electrophoresis studies and amino-terminal sequence analyses showed that the modified antithrombin obtained from either complex was identical to that produced in free form by the enzymes and also to the modified inhibitor that was shown previously to be released from the antithrombin-thrombin complex. The fact that identical findings were obtained for the reactions between antithrombin and three enzymes with different specificities strongly supports the previous proposal that the observed cleavage site is the active site of antithrombin.

0900

STUDIES OF THE HETEROGENEITY OF PURIFIED ANTITHROMBIN III. T.W. Barrowcliffe, C.A. Eggleton and M. Mahmoud. National Institute for Biological Standards and Control, London NW3 6RB, U.K.

Deficiency of antithrombin III (At III), whether hereditary or acquired, is now recognised as a major predisposing factor for the development of venous thromboembolism. Purified At III concentrates are undergoing clinical trials in various conditions associated with At III deficiency; such concentrates may be given in addition to heparin and their potency is usually assessed by heparin co-factor assays. In an international collaborative study, a reference preparation of purified At III had a lower concentration by heparin co-factor than by immunological assays and this was shown to be due to the presence of non-heparin-binding antigens. In the present study we have examined purified At III from several manufacturers by heparin co-factor (amidolytic), progressive antithrombin (clotting) and immunological assays, and their heparin-binding abilities have been studied by crossed immunoelectrophoresis and heparin-agarose affinity chromatography.

There was good agreement between progressive antithrombin and immunological assays, but in some concentrates the heparin co-factor assays gave lower activity. The proportions of non-heparin-binding material varied considerably, from less than 5% to as much as 50% of the total At III antigen in some concentrates. The non-binding material isolated from a heparin column had little heparin co-factor activity, but was able to neutralise thrombin and Factor Xa. Gel filtration and polyacrylamide gel electrophoresis showed no major distinction between heparin-binding and non-binding antigens, indicating the absence of At III-protease complexes.

These studies show that some At III concentrates contain substantial amounts of partially denatured molecules, in which the heparin-binding ability of the At III has been impaired but its thrombin and Xa neutralising activity left relatively intact.

0901

MECHANISM OF THE INACTIVATION OF TRYPSIN BY ANTITHROMBIN III. Å. Danielsson and I. Björk. Department of Medical and Physiological Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The reaction between bovine antithrombin III (AT) and bovine trypsin was studied and compared to the inactivation of thrombin by the inhibitor in order to elucidate general aspects of the mechanism of AT action. AT and trypsin formed inactive 1:1 complexes, as determined by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS/PAGE) and trypsin activity measurements. In the absence of heparin, the reaction was about 100-fold faster than the AT-thrombin reaction. The reaction rate increased when AT was preincubated with heparin before trypsin was added. Purified AT-trypsin complex dissociated at pH 10 into free enzyme and two proteolytically modified forms of AT, while no intact AT appeared. In SDS/PAGE, the two modified inhibitors gave bands corresponding to apparent molecular weights of 52000 and 48000 under reducing conditions, while both forms co-migrated with intact AT (mol wt 56000) under non-reducing conditions. This indicates that each of the two modified forms of AT had been cleaved into two or more chains held together by disulfide bonds. Under reducing conditions, the larger of the modified AT chains co-migrated grated with the large chain of thrombin-modified AT, i.e. the form of AT which dissociates from the AT-thrombin complex and which is cleaved at the reactive Arg-Ser bond of the inhibitor. Control experiments showed that the smaller of the two chains was formed by tryptic cleavage of the larger chain. Antisera specific for thrombin-modified AT reacted with purified AT-trypsin complex, demonstrating that the inhibitor was present in the complex in a form immunologically identical to thrombin-modified AT. An analogous finding has been reported earlier for the antithrombin- thrombin complex. Together, these results suggest the same general mechanism for inhibition of trypsin and thrombin by AT.

0902

ANTITHROMBIN III (ATIII) AND FIBRIN(OGEN) FRAGMENT E (FgE) IN DIABETIC NEPHROPATHY. Vivian Chan, C.K. Yeung and T.K. Chan, University Department of Medicine, Queen Mary Hospital, Hong Kong.

We measured plasma and urine ATIII and FgE levels by specific and sensitive radioimmunoassays (RIA) in 25 patients with diabetic nephropathy (DN) (proteinuria > 1g/day) and in 17 patients with non-diabetic glomerulonephritis (GN), matched for degree of proteinuria. Plasma ATIII in DN (mean ± SD, 19.37 ± 2.40 mg/dl) were lower than in diabetics without renal involvement (21.84 ± 2.86 mg/dl). Total urine ATIII was directly related to proteinuria and inversely to creatinine clearance. In GN patients, plasma ATIII levels were even lower (16.84 ± 3.78 mg/dl), but the amount of urine ATIII fell when creatinine clearance decreased to below 37 ml/min. Serum FgE levels were elevated in both groups and this was associated with increased total urine FgE excretion. In DN, serum and urine FgE were directly related to proteinuria but inversely to creatinine clearance, indicating an increase in intraglomerular fibrin deposition as the disease progressed. These findings suggest that in DN, intravascular thrombosis might play an intermediary role as mediator of glomerular injury. Furthermore, the monitor of urine ATIII and FgE reflected the severity of DN and could be useful indices of the progression of the disease.

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