0576
10:00 h

EFFECT OF HEPARIN ON THE THROMBIN-ANTITHROMBIN REACTION PRODUCT. Isidore Danishefsky and Michael S. Bender, Dept. of Biochemistry, New York Medical College, Valhalla, N.Y. 10595, USA

The characteristics of the primary complex (C-1) formed between thrombin and antithrombin in the absence and presence of heparin, were investigated. Each of the complexes were isolated by gel-filtration of the reaction mixture on Sephadex G-100.

Analyses by SDS-polyacrylamide gel electrophoresis showed that thrombin causes the successive degradation of both complexes to lower molecular weight products C-2 and C-3, respectively. C-1 that was formed in the absence of heparin also undergoes spontaneous direct degradation at pH 7.5, to a complex that is similar to C-3. Additionally, this C-1 dissociates very slowly to release thrombin, as demonstrated by its action on a synthetic substrate. Treatment of C-1 with 1M NH_2OH results in its breakdown to thrombin and antithrombin. The complex formed in the presence of heparin differs from the one formed without heparin, in that it does not exhibit any measurable dissociation and does not undergo breakdown to the C-3-type product. Moreover, whereas C-1 formed in the absence of heparin is decomposed completely by 1M NH_2OH, the complex formed in the presence of heparin undergoes only partial breakdown even with 2M NH_2OH. Addition of heparin to C-1 originally produced in the absence of heparin, has no effect on its properties.

The results thus indicate that heparin influences the mode of binding between thrombin and antithrombin as well as the rate of their interaction.

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10:15 h

HEPARIN EFFECTS (AND MECHANISMS) IN THE α_2-ANTITHROMBIN INACTIVATION OF SERINE PROTEASES--THROMBIN, PLASMIN, AND TRYPSIN. Gerald F. Smith and Jacqueline L. Sundboom. The Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN.

It is important to elucidate the effects of heparin on the α_2-antithrombin (ATIII) inactivation of serine proteases in order to understand the pharmacological activity of heparin. We have studied the enzyme kinetics of the ATIII inactivation of these proteases, and the effects of heparin on these interactions, using a common amide peptide substrate and protein substrates. We also studied the interactions of heparin with the three proteases.

We conclude that the mechanism of the catalytic effect of heparin (observed at 0.005 unit/ml) toward the thrombin-ATIII reaction is different from the mechanism whereby heparin (only at very high concentration, e.g., 10 unit/ml) can induce an enhanced rate in the plasmin-ATIII reaction. We conclude that the first mechanism involves a heparin-thrombin complex, while the mechanism with plasmin involves a heparin-ATIII complex which forms only at high heparin concentrations. This is consistent with known appropriate binding constants. We found that heparin has no effect on the very rapid inactivation of trypsin by ATIII. We further conclude that there is no common mechanism whereby clinically relevant levels of heparin cause general enhanced ATIII-protease reaction rates.

We suggest ATIII depletion during heparin therapy might be avoided by using low heparin levels, which would not allow heparin-ATIII complexes to form, yet which would catalyze the thrombin-ATIII reaction. Our finding that ATIII inactivates trypsin at a rate similar to the heparin-catalyzed thrombin-ATIII reaction suggests a physiological role for ATIII in the control of trypsin-like enzymes.

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10:30 h

ANTITHROMBOTIC PROPERTIES OF LOW MOLECULAR WEIGHT HEPARIN FRACTIONS FROM PORCINE MUCOSAL HEPARIN. J. Choay, Jean C. Lormeau, Harry L. Messmore, Jawed Fareed, J. Stulc and Anne Andersen, Loyola University Medical Center, Maywood, IL 60153 and Choay Institute, Paris, France.

A previous report from our laboratories has described the extraction and physicochemical properties of a low molecular weight fraction (mol wt 4-8 x 10^3 daltons) from porcine mucosal heparin (Choay et. al. thrombosis Res 18, 573, 1980). Beside exhibiting a strong anti Xa (>250 u/mg) activity, this product possessed strong antithrombotic properties in a modified rabbit stasis thrombosis model. At a 125 anti Xa u/kg it protected the thrombotic effects of activated prothrombin complex concentrate (20 u/kg) and Prothrombin Complex Concentrate/Russell's Viper Venom challenge in both the pretreatment and post-treatment regiments. At 1250 anti Xa u/kg SC it also showed antithrombotic effects for various periods. We have also obtained another low molecular weight fraction from porcine mucosal heparin by controlled depolymerization with nitrous acid. This product possessed saccharides with molecular weight ranging 3-6 x 10^3 daltons and exhibited a specific activity of >200 anti Xa u/mg. At a 125 anti Xa u/kg this product also showed antithrombotic activity against the thrombotic effects of activated prothrombin complex concentrates, prothrombin complex concentrates and Russell's Viper Venom. In contrast to these two low molecular weight fractions porcine mucosal heparin in identical anti Xa units failed to produce protection against the thrombogenic stimuli. Our studies suggest that low molecular weight heparin fractions with strong anti Xa and antithrombotic activities can be obtained by chemical depolymerization. Furthermore, their biologic properties are found to be similar to the naturally occuring low molecular weight fractions present in native porcine mucosal heparin.

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10:45 h

ANTIHEPARIN ANTIBODY: PREPARATION AND USE AS A FUNCTIONAL PROBE. S.N. Gitel, V.M. Medina, and S. Wessler. Department of Medicine, New York University School of Medicine, New York, N.Y., U.S.A.

Antiheparin antibodies were raised in 5 of 6 rabbits immunized sequentially with covalent complexes of ovalbumin-heparin and bovine IgG-heparin. Confirmation of the presence of heparin-specific antibodies in the antisera was based on: (1) antisera precipitation of BSA-heparin but not BSA alone, (2) antisera inhibition of heparin anticoagulant activity without precipitating heparin, (3) antisera precipitation of heparin-heparin complexes, and (4) antibody removal from the antisera by heparin-Sepharose. The antisera had hemagglutination titers between 1:10,000 and 1:50,000 when tested against tanned red cells labeled with BSA-heparin complex; whereas no hemagglutination occurred with tanned erythrocytes tagged with BSA. Heparin-heparin oligomers, prepared by carbodiimide coupling in aqueous solution and retaining anticoagulant activity, precipitated in the presence of the antiheparin antibodies. Data indicating that heparin has separate binding sites for antithrombin 111 and thrombin were obtained by quantitating the heparin oligomer-antibody precipitate in the presence and absence of these two purified, heparin-binding, coagulation proteins.

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