

POSTER PRESENTATIONS

11.30 – 12.45

 α_2 Antiplasmin – Experimental

Level 3 – Red Side I

Discussion Group 12.00 – 12.45

Poster
Board
P3-0010235 PURIFICATION OF α_2 -ANTIPLASMIN BY A SINGLE-STEP AFFINITY CHROMATOGRAPHIC PROCEDURE

B. Wiman, Department of Medical Chemistry, University of Umeå, Sweden.

A new and efficient single-step purification method for human α_2 -antiplasmin has been elaborated. The method is based on the interaction between α_2 -antiplasmin and a fragment (LBSI) constituting the three NH₂-terminal triple-loop structures in plasminogen produced by elastase digestion. This fragment has been purified and coupled to Sepharose and used for affinity chromatographic purification of α_2 -antiplasmin using plasminogen depleted plasma as starting material. After adsorption and washing at high ionic strength the α_2 -antiplasmin is specifically eluted with 6-aminohexanoic acid. The inhibitor preparation obtained in this way is over 90% pure as judged from SDS polyacrylamide gel electrophoresis and activity measurements. About 40-45 mg pure α_2 -antiplasmin per liter plasma is obtained representing a yield of about 60%. LBS-I Sepharose has much higher capacity for α_2 -antiplasmin and is also much more specific than plasminogen-Sepharose. Repetitive treatment of plasma with LBS I-Sepharose failed to adsorb the last 20% of α_2 -antiplasmin as judged by Laurell electrophoresis. This supports the recent finding of Clemmensen (1979) on partially purified α_2 -antiplasmin that a form of the inhibitor with less affinity for the lysine-binding sites in plasminogen may exist, even in unfractionated plasma. The major part of this type of α_2 -antiplasmin is also a functional antiplasmin since it can form a complex with plasmin.

P3-022 0236 FUNCTIONAL PROPERTIES OF TWO DIFFERENT MOLECULAR FORMS OF α_2 -PLASMIN INHIBITOR.

I. Clemmensen, S. Thorsen and S. Müllertz, Department of Clinical Chemistry, Hvidovre University Hospital, Copenhagen, Denmark.

The α_2 -plasmin inhibitor in plasma exists in at least two molecular forms (1). One form (PB) binds to plasminogen-Sepharose. The other form (NPB) comprising around 0.4 of the total amount of the inhibitor in plasma does not bind to plasminogen-Sepharose. PB was purified by affinity chromatography (1) while NPB was partially purified by conventional methods (2). Both inhibitor forms inhibited plasmin in a fast reaction and the change in catalytic concentration of plasmin with increasing concentrations of both forms was a linear function. At identical active site concentrations of the two forms (titrated with pNPG-titrated plasmin) PB produced a more marked inhibition of urokinase-induced fibrinolysis. Similarly PB produced a more marked decrease of the binding of Lys-plasminogen to fibrin than NPB. The results indicate that an equilibrium between the 2 forms may be important for the biological regulation of the inhibition of plasmin.

1: I. Clemmensen, The Physiological Inhibitors of Coagulation and Fibrinolysis; D.Collen, B.Wiman, M.Verstraete, eds., p. 131-136, Amsterdam 1978.

2: U.Christensen & I.Clemmensen, Biochem. J., 175:635-641,1978.