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POSTER SYMPOSIUM XV

Coagulation: Structure of Human Thrombin.

HUMAN α -THROMBIN PREPARATIONS. J.W. Fenton, II, J.S. Finlayson, M.J. Pasco, B.H. Landis, M.P. Zabinski, and D.L. Aronson. Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201 and Bureau of Biologics, Food and Drug Administration, Bethesda, MD 20014.

Thrombin production from human Cohn fraction III paste (>20 g; 340 ± 110 mg/kg paste, $N = 65$) has yielded predominantly α -thrombin ($97 \pm 3\%$, $N = 29$), with high clotting and esterase activities (2.8 ± 0.4 US "NIH" units/ μ g protein; $88 \pm 8\%$ esterase active, $N = 65$), free of detectable plasmin(ogen) and Factors II, VII, IX(a) or X(a). Preparations in 0.75 M NaCl ($\text{pH} \sim 6$) at 2-7 mg/ml ($A_{280\text{nm}} = 1.83 \text{ ml mg}^{-1} \text{ cm}^{-1}$, 0.10 M NaOH) retain activities frozen at $<-50^\circ\text{C}$ for >1 year. The enzyme (M_r 36,600 $\pm$ 800, $N = 9$) consists of two polypeptides (A chain M_r 4,600 $\pm$ 200, $N = 16$; B chain M_r 32,000 $\pm$ 1,000, $N = 22$), is an isoionically heterogeneous glycoprotein (pI 7.0, 7.3, 7.6; carbohydrate-staining B chain), displays sparing solubility (e.g., in 0.15 M NaCl, $\text{pH} \sim 7$; precipitates in H_2O), is easily denatured (low I; $\text{pH} < 5$ or > 10 ; $T_{1/2}$ 53°C), and autolyzes to non-clotting derivative forms (β - followed by γ -thrombin; both $< 1\%$ clotting of α -thrombin). Chloride salts (I 3, pH 8.3) of Group I (except Li) enhanced α -thrombin (1 mg/ml) thermal stability ($T_{1/2}$ 60-61 vs. 49°C for Li^+), whereas Group II salts reduced its stability ($T_{1/2}$ 40, 44, 48°C for Ca^{2+} , Ba^{2+} , Sr^{2+} , respectively). Autolytic loss of clotting activity (22°C , 48 h) followed the series: Na^+ ($> 90\%$ loss) $>$ K^+ (90%) $>$ Rb^+ (70%) $>$ Cs^+ (30%) $>$ Li^+ (10%) $\sim \text{Ca}^{2+} \sim \text{Sr}^{2+} \sim \text{Mg}^{2+} >$ Ba^{2+} ($< 1\%$). Clotting activity was correlated with enzymic homogeneity of α -thrombin (3.09 $\pm$ 0.57 US units/ μ g extrapolated pure-active enzyme, $N = 29$). (Supported in part by USPHS Grant HL 13160)

CHARACTERIZATION OF MULTIPLE FORMS OF HUMAN THROMBIN. J.J. Gorman and P.A. Castaldi, Haematology Department, Austin Hospital, Heidelberg, Victoria, Australia

Human thrombin was obtained by activation of partially purified human prothrombin with venom of the Australian Talpan (*Oxyuranus scutellatus scutellatus*).

The crude thrombin was precipitated with ammonium sulphate and subsequently purified by chromatography on Sephadex G-75 CM-Sephadex C-50 and the affinity resin aminobenzamidine-CH-Sepharose. The final preparation had a specific activity of 1700 units per absorbance unit ($A_{280\text{nm}}^{1\text{cm}}$) but was heterogeneous as shown by urea-acrylamide gel electrophoresis at acid pH and by isoelectric focusing. SDS-acrylamide electrophoresis revealed molecular weights of 39,000, 28,000, 25-23,000 and 15-12,000 for these proteins. The 39,000 dalton species predominated (greater than 90%) when the enzyme was inhibited with phenylmethanesulphonyl fluoride prior to dialysis against 0.02M sodium phosphate (pH 8.0) containing 0.1% SDS. Lack of such inhibition reduced the amount of the 39,000 dalton species to less than 60% with concomitant increase in the smaller species. Increase in the smaller species also occurred during incubation in 0.1M NaCl-0.1 M Tris buffer (pH 8.0).

Peptide mapping studies indicated that the smaller species were structurally related to the 39,000 dalton species. Amino acid compositions of tryptic peptides indicated a high degree of homology with bovine thrombin.

It has been established that human thrombin can exist in at least two secondary structural forms of different molecular weights, probably due to autolytic degradation of the largest (39,000 dalton) protein species.