

A SIMPLIFIED METHOD FOR PREPARATION OF HIGHLY PURIFIED HUMAN THROMBIN

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Increasing use of synthetic substrates for potency estimation of biological activities within the coagulation field has raised a demand for a supply of highly purified thrombin. We have simplified a thrombin preparation procedure using clinical factor IX concentrates as starting material. Activation was performed using fresh brain thromboplastin prepared in a simplified way. A first ionic exchange chromatography step was used, which got rid of 80% of the impurities. The thrombin was further purified on SP Sephadex comparatively following two different pathways. In one case the whole purification procedure was performed at pH 6.5 which led to a thrombin with proposed high stability but which gives a relatively low-yield preparation. In another purification procedure the preparation was performed at pH 7.4 which gives a considerably better yield but possibly poorer stability upon storage. In both cases a final purification step on heparin gel was performed. The pH 6.5 thrombin could be separated into two fractions, one with a specific activity of 2500 iu/mg and another with a specific activity of 6000 iu/mg. A protein peak corresponding to the highly active thrombin was obtained also from the pH 7.4 thrombin, but that material had lost its biological activity. The different thrombin preparations have been carefully ampouled and sealed under nitrogen in order to facilitate further stability studies and comparative studies on bio-assays using clotting methods as well as synthetic substrate methods.

NEW CHROMOGENIC PEPTIDE SUBSTRATE FOR FACTOR Xa. L. Aurell, R. Simonsson, S. Arielly, G. Karlsson, P. Friberger and G. Claesson, AB KABI, Peptide Research, Mölndal, Sweden.

The chromogenic substrate Bz-Ile-Glu-Gly-Arg-pNA is based on the primary structure preceding the bonds split by factor Xa in bovine prothrombin. Substitution of amino acids in this natural sequence by closely related amino acids has only given inferior substrates. Thus, the natural sequence seems very important. However, we have found that the free γ -carboxyl group of Glu is not indispensable. Substrates with the above structure but having been derivatized on the γ -carboxyl group of Glu, in the form of simple esters or amides, show improved properties. Especially Km of the new substrates compare favourably with our first substrate. Some of the amides also show increased Vmax.

These improved properties have made it possible to increase sensitivity, shorten incubation times and lower substrate consumption in several methods where this type of substrate is utilized.

A CHROMOGENIC SEMI-MICRO METHOD FOR THE DETERMINATION OF FACTOR Xa, FACTOR Xa INHIBITOR AND MINI-DOSE HEPARIN LEVELS. M. F. Scully and V. V. Kakkar. Thrombosis Research Unit, King's College Hospital Medical School, London.

Conditions influencing the measurement of heparin levels in the range 0 to 0.3 units/ml of plasma using the chromogenic substrate, Bz-Ile-Glu-Gly-Arg-pNA HCl (S2222, KabiVitrum Ltd) have been analysed to find the most suitable incubation mixture.

Factor Xa chromogenic activity was observed to be stable between pH 5.5 and 9.0 at 37°C. An optimal pH of 7.9 was found for the action of Factor Xa upon the substrate and also for the action of plasma inhibitor upon Factor Xa. A Michaelis constant (Km) of 0.6mM was determined for the hydrolytic cleavage of the substrate by Factor Xa. At a substrate concentration of 3 times Km, Factor Xa was measureable in the range of 0.02 to 0.12 units/incubation mixture with a change in optical density at 405 m μ of 0.06/second/unit corresponding to 5.7 n Kat/unit.

Using 100 μ l pooled plasma/unit of factor Xa, 55% of the Xa activity was inhibited at 3 minutes. Taking 3 minutes as a preincubation period, Xa inhibitor was measureable in the range 5 to 25 μ l normal plasma per 0.25 units of Xa with a net change in optical density at 405 m μ of 0.8. The greatest difference between the percentage inhibition of Factor Xa by heparinised (0.3 units/ml) and non heparinised plasma, however, was observed to occur after 1 minute preincubation. Using these observations a heparin calibration curve was constructed using 10 μ l aliquots of heparinised plasma. Heparin was measured in the range 0.06 to 0.3 units/ml plasma with a net change in optical density at 400 m μ of 0.8.