ASSESSMENT OF PLASMA FIBRINOLYTIC SYSTEM WITH CHROMOGENIC SUBSTRATE. Z.S. Latallo, E. Teisseyre and S. Lopaciuk, Institute of Nuclear Research and Institute of Haematology, Warsaw, Poland.

A block of tests employing a chromogenic substrate H-D-Val-Leu-Lys-pNA /S-2251, Kabi, Stockholm/ is proposed for a quantitative assessment of the plasma fibrinolytic system. High sensitivity of the substrate to plasmin makes it possible to assay the enzyme directly in plasma. Its activity remains unchanged during storage of plasma apparently due to the fact that the substrate is also sensitive to plasmin- α_2 macroglobulin complex. Introduction of conformational changes in plasminogen by acidification of plasma allows to complete plasminogen conversion into plasmin by streptokinase in 5 min. Both immediate and progressive antiplasmin activity is evaluated upon incubation of plasma with a standard plasmin preparation by measuring the residual enzyme activity /modif. method of Gyzander et al., Symposium "Neue Methoden der Gerinnungsanalyse", Freiburg, July, 1976/. Activator activity is expressed as plasmin activity resulting from human or bovine plasminogen after incubation with plasma. The whole set of tests can be performed within 30 min. using only 0.4 ml of plasma. Comparison of the results obtained from plasma samples of patients subjected to either continuous or intermittent treatment with streptokinase /alone or in combination with plasminogen/ indicate that this block of tests allows for a rapid and precise analysis of the pattern of the plasma fibrinolytic system in the course of treatment and may serve as a guidance in establishing the regime to be applied.

FLUOROGENIC SUBSTRATES FOR THE DETERMINATION OF PLASMINOGEN ACTIVATORS AND PLASMIN.

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Plasminogen activator activities in biological fluids are low and specific for plasminogen. Highly sensitive and accurate assays are needed for direct quantification and for kinetic studies. We therefore, synthesized tripeptide amides i.e., t. BOC. val. gly. arg β -NA (I) and val. gly. arg β -NA (II) from which the fluorescent group β -naphthylamine (β -NA) is released upon enzymatic hydrolysis.

Kinetic parameters found for the hydrolysis of these substrates by plasmin, urokinase and tissue plasminogen activator are:

	Plasmin		Urokinase		Tissue activator	
	I	II	I	II	I	II
K_m (mM)	1.6	0.9	1.1	0.6	0.5	-
V_{max}	2.4*	1.2*	3.0**	4.8**	2.4**	-

*in nmol/CU/min; **in pmol/CTA/min

Strikingly, tissue activator is not active on substrate II, allowing discrimination between this activator and other proteases.

The sensitivity of the assay is at least ten times higher than that observed for other synthetic substrates available. Moreover, substrates I and II can be used in enzyme histochemistry.

PHOTOMETRIC MEASUREMENT OF UROKINASE AND SIMILAR ACTIVITIES WITH A NEW ARTIFICIAL CHROMOGENIC SUBSTRATE. E. Wenzel, L. Svendsen, K.H. Nienhaus and L. Pfordt, University of the Saarland, Homburg-Saar, West Germany.

The sensitivity and the specificity of the photometric method for measuring urokinase and similar activities (thrombin, plasmin, trypsin, kallikrein) with an artificial chromogenic substrate (BZ - VAL - Gly - Arg - PNA = UK - Chromozym) were evaluated. For reference, a urokinase from Abbott Laboratories (Lot No. 143/291 Abbott, North Chicago, Illinois) was used. For a final concentration of UK - Chromozym of 0.1×10^{-3} , 1 mU UK - Chromozym was found to be adequate to 17.2 U (Abbott). For 0.6×10^{-3} M, 1 mU UK - Chromozym was found to be in accordance to 10.4 U (Urokinase Abbott). In a preparation of 1 NIH thrombin (Boehringer Mannheim Diagnostica), an activity of 0.82 mU UK - Chromozym was measured. In plasmin (human, Kabi München U), 84.8 mU UK - Chromozym were detected (1 Casein U per ml = $0.848 \Delta OD/min.$). For 1 NF trypsin, 19.7 mU Urokinase Chromozym were measured ($1 \Delta OD/min. = 0.197$). For comparing the different activities in commercially available urokinase preparations (Seren, Heidelberg, Urokinase Green Cross, Osaka), a Tris-Imidazol buffer was used (ionic strength 0.15; pH 8.4; containing 100 mg albumin, bovine per 1 and 10 U Aprotinin per 1). Thus, unspecific activities similar to urokinase activity were blocked (plasmin, trypsin, kallikrein, etc.). The same system was used for measuring the anti-urokinase-activity in the plasma of 25 healthy human volunteers and in 21 patients suffering from arterial occlusive diseases or from venous thrombosis before, during and after fibrinolysis therapy and before and after treatment with anticoagulants. These first results of clinical investigations will be discussed.