

Friday, July 17, 1981

Symposium XVI

Chromogenic Substrates

15:30-17:30 h

Cinema 1

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THE USE OF PEPTIDE FLUOROGENIC SUBSTRATES IN CYTOCHEMISTRY. R.E. Smith. Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507, L-452, Livermore, California, USA 94550.

Cytochemical demonstration of proteases by fluorescent techniques is only a recent achievement. Localization of cellular proteases through the use of synthetic peptide substrates (as opposed to use of antigen-antibody in immunocytochemistry) depends upon the enzyme-substrate complex theory which assumes combination of enzyme with substrate in phase I and liberation of the enzyme and products in phase II. Determination of intracellular protease activity depends on liberation of a fluorophore attached to the peptide by an arylamide or ester bond. To localize the site of enzymatic hydrolysis of the substrate, the product must remain immobilized. Overcoming diffusion of reaction product is not a new problem in chromometric detection of enzymes, but it is enhanced with fluorometric detecting systems. Fluorescence microscopy allows for more precision in localizing reaction product, therefore diffusion is more noticeable. In flow cytometry, monodispersed cells are in a liquid transport media; thus diffusion of reaction product from a cell is unacceptable for it may be entrapped by another cell.

Although peptide derivatives of naphthylamines have been used in cytochemistry as chromogenic substrates for over 20 years, their fluorogenic potential was not useful until development of the concept of wavelength shifting. Naphthylamines fluoresce at short UV wavelengths, so the reaction product is difficult to quantitate above background interference. Furthermore, the product is soluble and easily washed out of cells or tissue. Coupling of 4-methoxy-2-naphthylamine to 5-nitrosalicylaldehyde provides a method of reaction product immobilization as well as extending the emission wavelength into the yellow-orange region of the spectrum. Nevertheless the technique has certain limitations, unavoidable in a two-step reaction product capture method. Recently, we synthesized peptide derivatives of several types of coumarins; some are proving to be useful fluorogens in the cellular localization of proteases.

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AMINO ACID AND PEPTIDE THIOESTERS: NEW SENSITIVE SUBSTRATES FOR BOVINE FACTORS IX_a, X_a, XI_a, XII_a, THROMBIN, PLASMA KALLIKREIN AND TRYPSIN.² James C. Powers and Brian J. McRae. School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA.

A series of amino acid and peptide thioesters have been synthesized as sensitive assay substrates for the serine proteases involved in blood coagulation. Each substrate had a P₁ Arg residue. The thiol leaving group (P₁') and the P₂ amino acid residues were varied. The thioesters have much higher K_{cat}/K_m values than the corresponding amides such as 4-nitroanilides and 7-amino-4-methylcoumarin amides. In addition, the thiol released upon enzymatic hydrolysis of the substrates, can be measured chromogenically by use of a thiol reagent such as 4,4'-dithiodipyridine contained in the assay mixture. Thioesters are among the most sensitive assay substrates for coagulation serine proteases due to the combination of a high turnover rate with the ease of detection of the hydrolysis product. In addition they can be utilized with enzymes such as factor IX_a for which no other suitable synthetic substrate is currently available.

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CLINICAL INFORMATION FROM SCREENING PROGRAMS FOR COAGULATION AND FIBRINOLYTIC FACTORS AND INHIBITOR DEFICIENCIES USING AUTOMATED CHROMOGENIC METHODS. J.W. ten Cate, L.H. Kahlé, H.R. Büller, M. Peters, G.H. Weenink. Department of Hematology, University Hospital "Wilhelmina Gasthuis" Amsterdam, The Netherlands.

The introduction of chromogenic substrates allowed the development of automated spectrophotometric assays. After having developed such methods employing an automated kinetic enzyme and substrate analyser for coagulation factors II and X, anti-thrombin III (AT III), plasminogen (PG) and α_2 -antiplasmin (α_2 -AP), we decided to apply these methods in well defined clinical projects in order to evaluate their future clinical potency. Results obtained thus far revealed that 1. AT III and PG are predictors of gram negative septicaemia in patients with intraabdominal infection following major abdominal and vascular surgery, 2. The predictive value of AT III for gram negative septicaemia in prospective studies in premature neonates (all assays require only 0.4 ml of venous blood), 3. AT III remains unchanged in normal pregnancy. Decreasing values are observed in toxæmia and isolated deficiencies are observed as an early sign of intravascular coagulation in pre-eclampsia, 4. Routine screening of AT III in female subjects on oral anticonceptive drugs is of no use, 5. Factor X assays may possibly be used for the control of oral anticoagulant therapy, 6. Screening of patients with postoperative bleeding disorders may disclose congenital factor X deficiency not reflected by routine prothrombin time measurements.