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Note

Use of fluorescamine in peptide mapping on thin-layer cellulose plates

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Fingerprinting of peptide mixtures obtained by proteolytic digestion of normal and variant proteins provided a simple technique for the identification and isolation of defective peptide sequences[1]. Peptide mapping has also been used in studies of the sub-unit composition of complex protein molecules. The protein is usually hydrolyzed by a proteolytic enzyme and the resulting peptide mixture fractionated by a combination of high-voltage electrophoresis and chromatography on filter paper or thin-layer plates. The resolution of spots is limited by the presence of larger peptides which are resistant to digestion and often insoluble. In our work on the sub-unit composition of fibrinogen, we were confronted with the problem of detecting over 100 spots on a single peptide map. In order to localize all these peptides by the conventional ninhydrin reaction, we had to apply relatively large amounts of digested material, and consequently the resolution of spots was poor owing to smearing. Therefore, we tried the spray reagent fluorescamine, which has already been used for the detection of amino acids on thin-layer silica gel plates[2].

A representative peptide map of the fragment d of fibrinogen[3] is presented in this paper. Protein (1 mg) was dissolved in 0.2 *M* ammonium hydrogen carbonate of pH 8.5 and incubated with 20 μg of TPCK *-treated trypsin for 4 h at 37°. The digest was lyophilized and the dry residue dissolved in 50 μl of distilled water. Aliquots of 5 μl were applied on glass plates (20 × 20 cm) covered with a 0.5-mm layer of Whatman CC-41 cellulose. Electrophoresis was performed at pH 5.5 in a pyridine–acetic acid–water (20:7:973) buffer[4], using methyl-green as a marker. This dye was found to have a mobility comparable with that of arginine. The running time was, on average, 70 min at 1000 V. After electrophoresis, the plates were dried for 30 min at 80° and then subjected to ascending chromatography in *tert.*-butanol–formic acid–water (695:10:295)[5]. After drying for 30 min at 80°, the cold plates were sprayed with 0.05% fluorescamine (Fluoram; Roche, Basel, Switzerland) in acetone and viewed or photographed after 30 min under long-wave ultraviolet light. Subsequently, the plates were sprayed with 0.2% ninhydrin in acetone and heated for 5 min at 80°. Photographs taken on Polaroid film (Type 107) are shown in Figs. 1 and 2. Fluorescamine staining reveals at least 35 spots, whereas only 20 spots can be detected with ninhydrin. The ratio of the staining intensities is specific, which facilitates

* TPCK = L-1-tosylamide-2-phenylethylchloromethyl ketone.

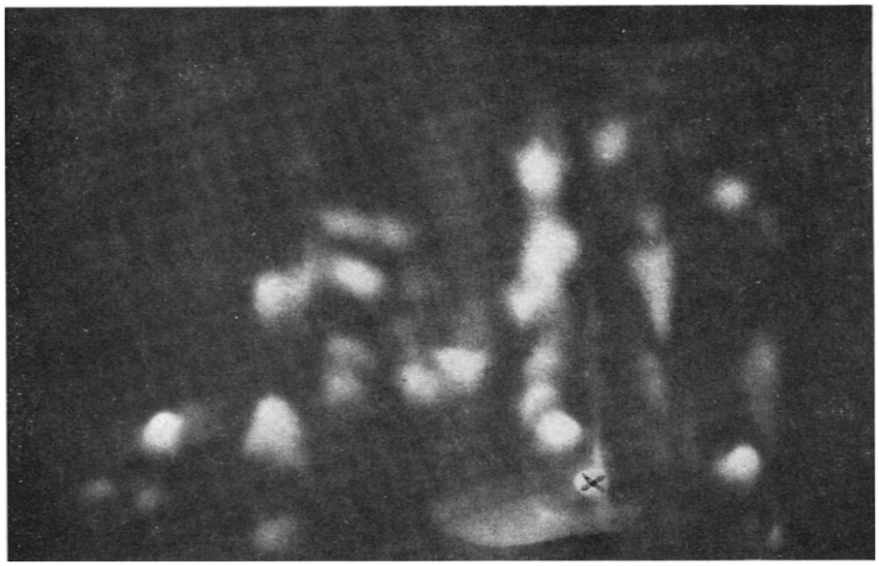

Fig. 1. Thin-layer peptide map of the tryptic digest of fibrinogen fragment d. The plates were sprayed with fluorescamine and photographed under ultraviolet light. The cross denotes the point of sample application before electrophoresis.

the identification of the individual peptides on fingerprints. Using the method of peptide mapping, the sub-unit composition of the plasmin-resistant disulphide knot in fibrinogen fragment d has been determined. These results will be published elsewhere.

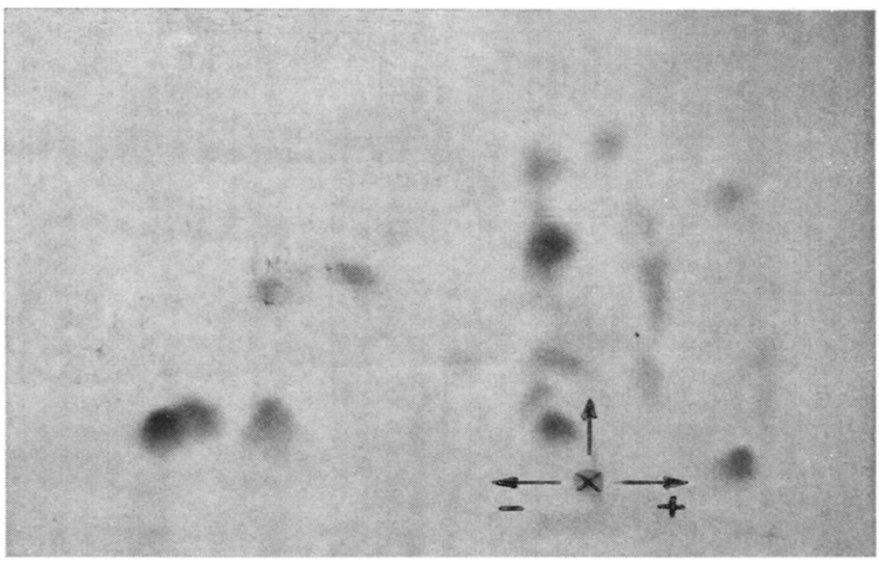

Fig. 2. Same plate as in Fig. 1, additionally sprayed with ninhydrin.

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