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An improved method for peptide mapping

The number of peptide spots in maps of large proteins may well be over one hundred. In the course of our work on bovine fibrinogen it became obvious that the resolution of standard electrophoresis–chromatography procedures for obtaining peptide maps (see for example KATZ *et al.*[1]) is inadequate for this purpose. The 46 × 57 cm chromatographic paper was not large enough to distinguish all the peptides. Our preliminary attempt to resolve this difficulty involved the use of a larger sheet of paper (46 × 150 cm). The sample spot was applied 45 cm from the positive electrode, so that the basic peptides had to move up the paper, over the supporting bar, and down the other side, towards the negative electrode. The resulting peptide maps showed a decided smearing of the basic spots and were not reproducible (Fig. 1). This smearing was attributed to the difficulty in uniformly blotting the excess buffer from the large sheet of paper, and the establishment of a potential gradient at the supporting bar, as the buffer drained down the paper. The basic peptides, upon traveling across this potential gradient, were extended into streaks.

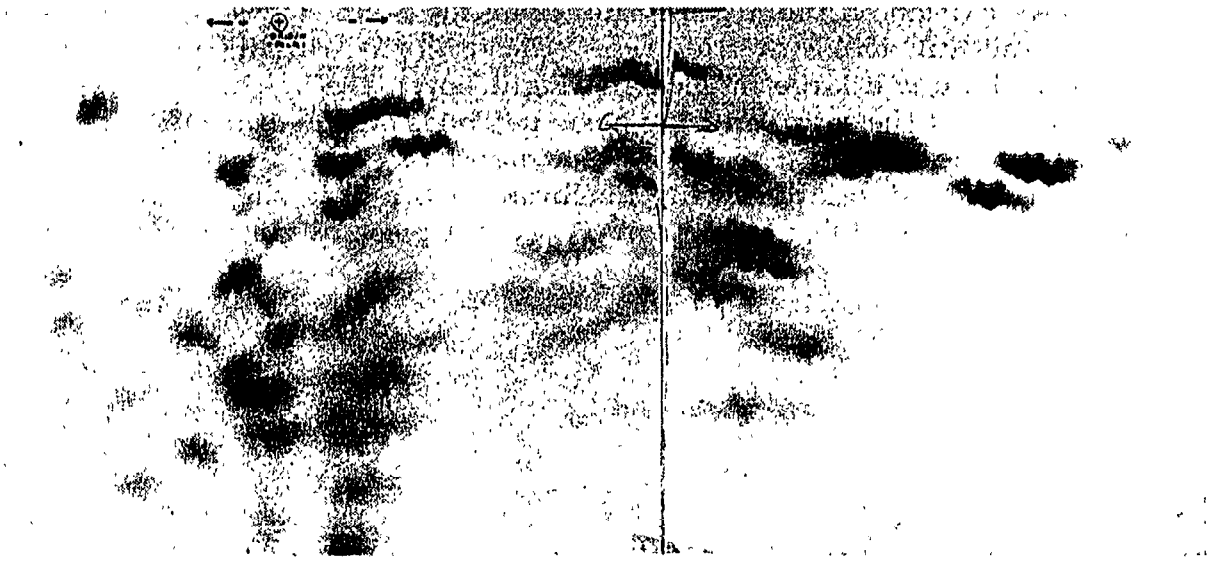

Fig. 1. Peptide map of a large tryptic fragment of bovine fibrinogen, which contains about 60% of the peptide spots of the whole molecule.

To counteract these difficulties a new technique in electrophoresis was developed which gives better resolution and allows several experiments to be performed concurrently. The main features of this technique will be described in this communication.

The samples were prepared as follows: The protein was sulfitolyzed in 5 *M* guanidine HCl solution using either the method of NIELSEN *et al.*[2], or that of PECHÈRE *et al.*[3]. The solution after sulfitolysis was dialyzed free of salts and either used directly, or lyophilized and made up to a 1% solution in water, adjusting the pH to 9.0–9.5. Dissolution was usually complete, or the small amount of insoluble material went into solution during the digestion. Digestion was performed by trypsin added in the amount

of 1 % of the protein weight. Salt-free, twice crystallized trypsin from Worthington Biochemical Corp., Freehold, N.J. (TRL 6JA) was used in a 1 % solution in 0.001 *M* HCl*. The digestion was continued for 18 h at room temperature (25°), while the pH was maintained at 8.5 by a pH-stat. The digest was lyophilized and then made up to a 100 mg per ml solution in water, using dilute NaOH to adjust the pH to approximately 9.

The high-voltage electrophoresis was run in a pyridine–glacial acetic acid–water solution (30:10:960) at pH 5.4[6]. Whatman No. 3 MM chromatographic paper was cut into strips 2.5 cm wide and 150 cm long. Between 100 to 160 μl of sample was applied as a band across the middle of the strip in 20 μl aliquots. The strips were wet with buffer and blotted to remove excess liquid. A homemade rack, similar to the one described by KATZ *et al.*[1], was used to support the strips during the experiment, such that the sample was in the center over the supporting bar and the two ends of the strip hung to either side. As many as eight strips were run at the same time. The electrophoresis was performed in a Model D, high-voltage electrophorator, Gilson Medical Electronics, Middleton, Wisc., filled with varsol (a light petroleum fraction made by Standard Oil Company of New Jersey) and cooled by running tap water. The potential difference was 5000 V, and the current varied between 8 and 10 mA per strip. After the current had been on for 3.5 h, the electrophoresis was terminated, and the strips were dried on the rack in a 60° oven for 2–3 h. The strips were removed from the rack and cut across at the origin (the origin is usually devoid of peptide material, unless there is a large molecular weight core), the acidic peptides now positioned on one half of the strip, and the basic peptides on the other. Each half of the strip was sewn to the edge of a piece of Whatman No. 3 MM chromatography paper (46 × 57 cm) using a standard sewing machine with a "zig-zag" stitch, such that the strip overlapped the paper by 0.5 cm. A second piece of chromatography paper

Fig. 2. Peptide map of bovine fibrinogen. On the originals 145 reproducible spots were counted.

* This enzyme preparation had 1.8 % chymotryptic activity when tested on a standard tyrosyl ethyl ester solution[4]. The small amount of chymotrypsin present (the actual amount may be even smaller because the estimate does not take into account the cross reaction of these enzymes with each others substrate) does not seem to present problems, since an identical peptide map was obtained with a trypsin preparation in which chymotrypsin was inhibited by N-tosyl-L-phenylalanine chloromethyl ketone[5].

(9 × 57 cm) was sewn to the other edge of the strip, again so that the strip overlapped. All these operations should be performed with rubber gloves. Descending chromatography was then performed for 18 h with pyridine–1-butanol–acetic acid–water (100:150:30:120) buffer of pH 5.1[7]. Upon completion of the chromatography, the paper was dried in the 60° oven and stained by dipping it in buffered 0.3 % ninhydrin in acetone solution[8].

By placing the origin on the bar, the difficulty of the basic peptides traveling through the potential gradient was eliminated. The strips allowed for easier handling, more uniform blotting, and multiple concurrent runs. Fig 2 shows the peptide map of bovine fibrinogen on which up to about 135 spots were counted. The maps are easily reproducible, with well resolved, round, undistorted spots.

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