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High resolution techniques of peptide mapping. Separation of bovine carotid actin peptides on cellulose thin layers and of the corresponding dansyl-peptides on polyamide thin layers

Comparative studies of globular actin, extracted from smooth or skeletal muscles from different animals, have failed in general to reveal significant differences in amino acid composition[1]. Bovine carotid actin is not an exception in this respect, its amino acid composition being similar to that of actin from rabbit skeletal muscle[2]. The same uniformity also occurs in the case of their peptide maps. Previous investigations on tryptic digests of carboxymethylated actin, performed by peptide mapping[3,4] after paper chromatography and electrophoresis, revealed about thirty ninhydrin positive spots. Using elaborate combined techniques of column chromatography and high voltage paper electrophoresis, MARTONOSI[5] detected forty-one peptides. On the basis of arginine and lysine content and for an assumed molecular weight of 60000[6,7], more than fifty peptides are theoretically liberated during trypsin digestion, whereas about forty would be expected for a molecular weight of 47000, the latter having been proposed more recently[4,8]. The number of peptides obtained on paper maps is insufficient evidence for determining minor variations appearing in the primary structure of actin extracted from different sources. The technique of MARTONOSI, even if it does allow an approach to the theoretical figures, is too complicated to be useful in comparative studies. For this reason, we undertook an extensive study in order to develop simple techniques, whereby improved resolution of the tryptic peptides of G-actin could be achieved. One hundred and fifty tests were carried out by means of two-dimensional techniques on thin layers of cellulose, silica gel and polyamide, using more than fifty chromatographic mixtures. Two techniques were finally selected: the first makes use of a cellulose thin layer, and allows detection of 46 ninhydrin positive spots, whereas the second, using dansyl-peptides, reveals 44 U.V. fluorescent spots on a polyamide layer.

Materials

Bovine carotid actin has been prepared by GASPAR-GODFROID *et al.*[9], according to the method of CARSTEN AND MOMMAERTS[10]. The carboxymethylation of the protein was performed with iodoacetate in 8 *M* urea in the presence of β-mercaptoethanol[11] whereas the tryptic digestion of the carboxymethylated actin was carried out as described by GOSSELIN-REY *et al.*[2].

Cellulose MN 300 (Macherey, Nagel, Düren, Germany) was used to prepare the thin layers.

Polyamide layers were purchased in 15 × 15 cm sheets from Cheng Chin Trading Co. Ltd., Taipei, Taiwan.

Dansyl chloride (98 %) was obtained from Fluka A.G.

Other chemical reagents were "Analytical grade". Note that the pyridine (Merck No. 9728) was redistilled on ninhydrin (500 ml pyridine–1 g ninhydrin) to avoid a coloured background.

Experimental

The peptide map on a cellulose thin layer. Standard 20 × 20 cm glass plates are spread (Desaga Equipment) with a 500 μ layer of cellulose (32 g cellulose, 192 ml water, 8 ml ethanol, stirred 2 min in a Waring blender). The plates are dried overnight in a cupboard at ordinary temperature and then washed with the chromatographic solvent; after washing, the upper yellow band is scraped off and 15 μl of peptide solution (5 mg/ml) is applied 10 cm from one edge and at 2 cm from the bottom. The plate is equilibrated as described by BONDIVENNE AND BUSCH[12], for 30 min in the chromatography tank, and then developed with amyl alcohol–isobutanol–propanol–pyridine*–water (10:10:10:30:30) for 6 h.

After drying the plate at ordinary temperature, electrophoresis is started at right angles to the first direction using pyridine–acetic acid–water (300:10:2700) at pH 6.5. The temperature of the cooling plate of the Desaga apparatus is kept at 5° with the aid of a circulating bath. After 50 min at 1000 V (20 mA) the plate is dried and then dipped in a ninhydrin reagent (1 g ninhydrin, 700 ml ethanol, 20 ml 2,4,6-collidine, 210 ml glacial acetic acid). The plate is heated at 110° for 15 min in an oven; 46 violet or gray coloured spots appear on a white background (Fig. 1).

The dansyl-peptide map on a polyamide layer. The dansylation of the peptides is performed as described by GRAY[13]. Peptides (0.5 mg) are dissolved in 50 μl 0.2 *M*

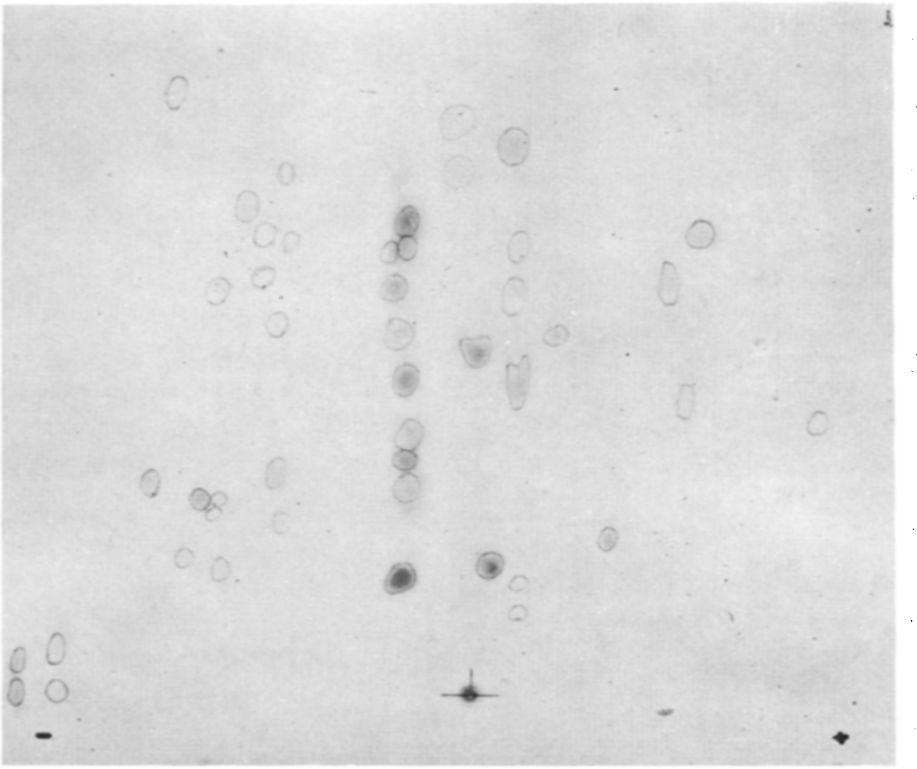

Fig. 1. Map of the peptides of a tryptic digest of bovine carotid actin on a 500 μ thin layer of cellulose. First direction (bottom to top): Chromatography in amyl alcohol–isobutanol–propanol–pyridine–water (10:10:10:30:30); second direction: Electrophoresis at pH 6.5 in pyridine–acetic acid–water (300:10:2700) for 50 min at 1000 V (20 mA).

* See section Materials.

$NaHCO_3$, 60 μl of an acetone–dansyl chloride solution (5 mg/600 μl) are added and the mixture, at pH 9, is incubated at 37.5° for 1 h.

After evaporation *in vacuo*, the residue is redissolved in 50 μl of deionized water and the dansylation process repeated twice more; care being taken to readjust the pH to 9 by addition of 0.1 *N* NaOH and to avoid any precipitation by adding acetone.

The mixture is then evaporated to dryness and 1 ml of 0.01 *N* acetic acid is added. This solution is passed through a column of Dowex 50 X 2 H^+ (7 × 0.9 cm), previously equilibrated with 0.01 *N* acetic acid[14]. The bulk of the by-products is eliminated by washing with 150 ml of 0.01 *N* acetic acid and the dansyl-peptides are eluted with a 25 % acetone solution in 1 *M* NH_4OH. U.V.-fluorescent fractions are pooled and evaporated to dryness *in vacuo*. The residue is dissolved in 500 μl of an acetone–acetic acid mixture (50:50) and the solution is kept at 2°.

To resolve the dansyl-peptides, 5 μl of the solution is applied to the right corner of a 15 × 15 cm plate of polyamide thin layer. The plate is developed first with a chromatographic solvent proposed by WOODS AND WANG[15] (see legend of Fig. 2) for 1 h; after drying, a second development is carried out at right angles using the following mixture, xylene–pyridine–acetic acid (10:1:1), for 2 h.

After drying, the chromatogram is exposed to a U.V.-lamp; 44 fluorescent spots were detected (Fig. 2).

This method, using dansyl-peptides and a polyamide layer, can be compared favourably with the other one in view of its simplicity, its low cost and its twenty times higher sensitivity. However, great care must be taken in the peptide-labelling process to avoid incomplete reaction which can provide additional spots.

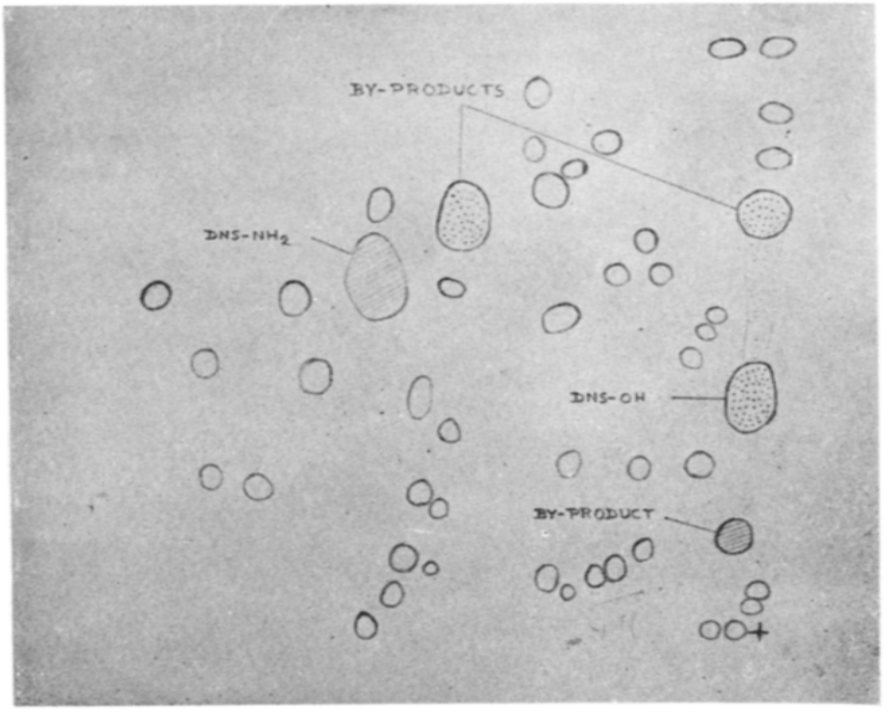

Fig. 2. Map of the peptides of a tryptic digest of bovine carotid actin on a polyamide layer using dansyl-peptides. First direction (bottom to top): Chromatography in water–formic acid (200:2.7); second direction (right to left): Chromatography in xylene–pyridine–acetic acid (10:1:1). Dotted area: blue fluorescent spots. Striated area: strongly yellow fluorescent spots.

Conclusions

An increase of about 30% over the number of peptides detected in G-actin with previously reported techniques of peptide mapping on paper is obtained by means of two simple thin layer techniques described in this paper. The number of spots detected is close to the maximal value theoretically expected and thus indicates that G-actin must consist of either one unique[8] or two different polypeptide chains and not of two very similar subunits as proposed by JOHNSON *et al.*[4].

The present results suggest that both techniques might be advantageously used, independently or together, to reassess the so-far observed similarity of G-actin extracted from different sources, as well as to undertake comparative studies on any homologous proteins.

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Identification and quantitation of α-hydroxy and α-keto acids with a ceric ammonium nitrate reagent

The use of ceric ammonium nitrate for the identification of α-hydroxy acids, α-keto acids and mercaptans in paper chromatograms was reported[1]. In continuation of the above, the reagent has now been stabilized and modified for thin-layer chromatograms. A quantitative procedure has also been developed.