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Note

A new solvent system for the resolution of all common Dns amino acids on polyamide plates

O. BERTRAND, Y. KROVIARSKI and P. BOIVIN

INSERM U160, Unité de Recherches d'Enzymologie des Cellules Sanguines, Hôpital Beaujon, 92118 Clichy Cédex (France)

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Derivatization of amino acids with free amino groups with 5-dimethylamino-naphthalene-1-sulphonyl(dansyl)chloride made it possible to devise very sensitive methods for N-terminal end-determinations in proteins and for manual Edman degradations[1]. In addition, this reagent has also been used for the most sensitive method of quantitative amino acid analysis[2,3].

The identification of dansylated amino acids (Dns amino acids) can be carried out by a number of methods but two-dimensional thin-layer chromatography on polyamide plates, introduced by Woods and Wang[4], has gained widespread use because of the rapid and accurate resolution of these derivatives.

Many solvent systems have been described for the chromatography of Dns amino acids on polyamide plates[1,4,5], but as far as we know, with every solvent system for complete resolution of all Dns amino acids to be achieved, more than two runs are necessary.

We describe here a solvent system that gives, with only two runs a clear separation of all common Dns amino acids.

EXPERIMENTAL

Materials

Dansyl chloride was purchased from Calbiochem (Los Angeles, Calif., U.S.A.), pure proteins, polylysine and free amino acids from Sigma (St. Louis, Mo., U.S.A.), and standard Dns amino acids from Nutritional Biochemical Corp. (Cleveland, Ohio, U.S.A.). Dns carboxymethylcysteine was prepared from di-Dnscystine; O-Dns tyrosine and ε-Dns lysine were prepared by dansylation followed by acid hydrolysis of succinylated bovine serum albumin for the former and synthetic polylysine for the latter. Dns methionine sulphone and sulphoxide were prepared from the parent amino acids. All other chemicals were reagent grade products from Merck (Darmstadt, G.F.R.). Polyamide plates were obtained from Schleicher and Schüll (Dassel, G.F.R.).

Chromatographic tanks were made from 1-l beakers cut 12 cm from the bottom and closed with a glass plate. The chromatographic plates were held standing in a vertical position by a home-made all-glass holder.

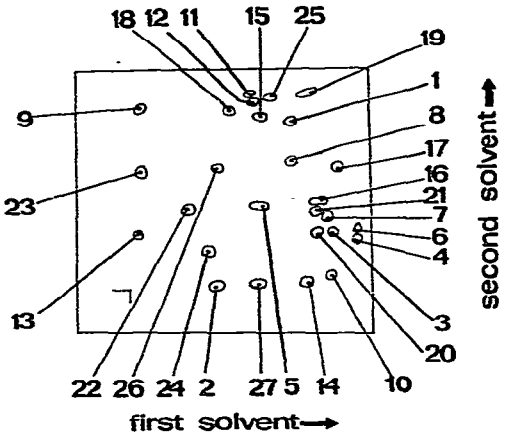

Fig. 1. Migration of all Dns derivatives studied. 1 = Dns Ala; 2 = Dns Arg; 3 = Dns Asn; 4 = Dns Asp; 5 = Dns carboxymethylcysteine; 6 = Dns Glu; 7 = Dns Gln; 8 = Dns Gly; 9 = Bis-Dns Tyr; 10 = α-Dns His; 11 = Dns Ile; 12 = Dns Leu; 13 = Di-Dns Lys; 14 = ε-Dns Lys; 15 = Dns Met; 16 = Dns methionine sulphone; 17 = Dns methionine sulphoxide; 18 = Dns Phe; 19 = Dns Pro; 20 = Dns Ser; 21 = Dns Thr; 22 = Dns Trp; 23 = Di-Dns His; 24 = O-Dns Tyr; 25 = Dns Val; 26 = Dns amide; 27 = dansic acid.

Solvents

The first solvent was prepared by weighing 80 mg of ammonium chloride, adding 22 ml of ammonia solution (0.91 per 50 g), 10 ml of absolute ethanol and distilled water to 100 ml.

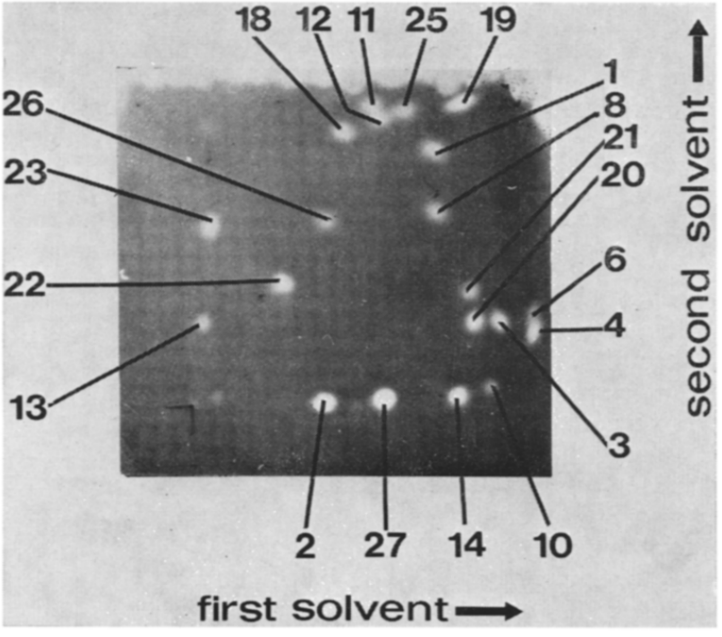

Fig. 2. Two-dimensional chromatography of some Dns amino acids on polyamide plate. Numbering as in Fig. 1.

The second solvent was benzene–pyridine–acetic acid (75:2:6, v/v/v). An analogous formulation has been described for the chromatography of Dns amino acids on silica plates[6].

Procedure

Polyamide plates (5 cm × 5 cm) were used. A dansyl amino acid mixture in ethanol was carefully spotted with a finely drawn microlitre pipette on to the origin 9 mm from the edges; the use of a hair dryer made it possible to obtain a very tiny spot (1 mm diam.). Ascending chromatography was carried with first solvent. When the solvent front had reached the upper edge of the plate, the plate was thoroughly dried with a hair dryer and development with the second solvent was performed at right angles. After drying, the plate was viewed under a UV lamp.

RESULTS AND DISCUSSION

Excellent resolution of Dns amino acids was obtained (Figs. 1 and 2) and there is no need for a third run. However, it must be emphasized that such good results can be obtained only if care is taken with the spotting of Dns amino acid on to the plate, otherwise overlapping of critical pairs (*e.g.*, Dns aspartic acid and Dns glutamic acid) is observed.

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