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Note

Improved solvent system for thin-layer chromatography of Dns-amino acids

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Coupling of the Edman degradation with dansylation of N-terminal residues has become increasingly popular as a technique of manual peptide sequencing. Numerous methods have been developed for the separation of Dns*-amino acids (for a review see refs. 1 and 2) among which thin-layer chromatography (TLC) is the most widely applied, because of its sensitivity[3–5]. There are several solvent systems for two-dimensional chromatography of Dns-amino acids[3,6–8]. Those developed by Woods and Wang[7] and modified by Hartley[3] offer the least ambiguity, because the Dns-amino acids are widely distributed over the two-dimensional map. However, after two-dimensional chromatography many Dns-amino acids remain unresolved, and an additional chromatographic step with a third solvent is often required. For example, the pairs Dns-Asp/Dns-Glu, Dns-Thr/Dns-Ser and Dns-Ala/Dns-NH_2, and the quartet Dns-His/Dns-Arg/Dns-α-Lys/Dns-ε-Lys, are only separated after a third solvent, ethyl acetate–methanol–acetic acid, is applied[3]. In this note, we describe a modified second solvent, with which the need for a third solvent can be practically eliminated.

EXPERIMENTAL

Materials

Chen-Chin polyamide sheets were obtained from Gallard-Schlesinger, Carle Place, N.J., U.S.A. Dns-amino acids were purchased from Pierce, Rockford, Ill., U.S.A., with the exception of Dns-Met and Dns-carboxymethylcysteine which were prepared as described by Gros and Labouesse[8]. All other chemicals were of analytical-grade purity.

The solvents used were: I, 1.5% formic acid[7]; II_M (modified solvent II[7]), benzene–acetic acid (4.5:1), to run at right angles to solvent I. In the original solvent II[7] the benzene:acetic acid ratio was 9:1. On some special occasions (see Results and discussion), two parts of ethyl acetate were added to ten parts of solvent II_M.

Chromatography

Mixtures containing 100 pmoles of each Dns-amino acid were applied to a

* Dns = dansyl = 5-dimethylaminonaphthalene-1-sulfonyl.

point *ca.* 0.7 cm from the lower left edge of 5 × 5 cm polyamide sheets. Each plate was developed in a 150-ml beaker containing 15–20 ml of solvent and covered with an inverted 400-ml beaker. The chromatography was stopped when the distance travelled by the solvent front was *ca.* 3.5 cm. Development with solvent I took 3–4 min, and with solvent II_M *ca.* 5–7 min. The entire procedure, including application of sample and thorough air drying between application of the two solvents, requires less than 15 min. Spots of the Dns-amino acids which were fluorescent were located under a long-wavelength UV lamp.

RESULTS AND DISCUSSION

Fig. 1A represents two-dimensional chromatography with solvents I and II_M. In contrast to the original method[3,7] (Fig. 1B), the pairs Dns-Asp/Dns-Glu and Dns-Thr/Dns-Ser were clearly resolved without loss of resolution of other Dns-amino acids. Dns-His was separated from the still unresolved Dns-α,ε-Lys and Dns-Arg. Dns-carboxymethylcysteine was also separated from Dns-OH, although Dns-cysteic acid may still be obscured by the large, intensely fluorescent, Dns-OH. Except for the separations of Dns-Ala from Dns-NH_2 and of Dns-Arg from Dns-α- or ε-Lys, no third solvent was required. Dns-Ala and Dns-NH_2 exhibited fluorescent spots of different color, and sometimes they could be differentiated on this basis without further chromatography. When necessary, Dns-Ala and Dns-NH_2 could be readily separated with solvent III of Hartley[3] after application of solvents I and II_M, or by chromatography with ethyl acetate in solvent II_M (see Experimental) after solvent I.

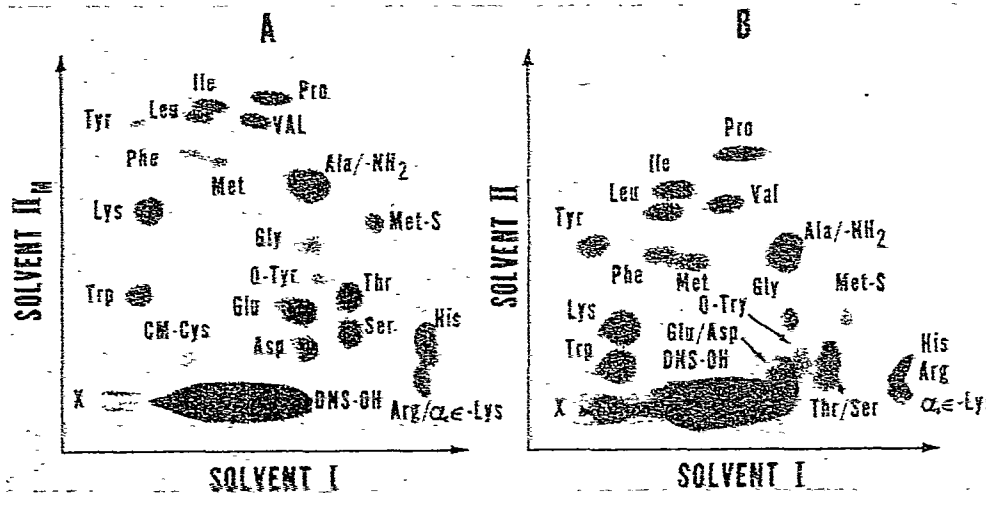

Fig. 1. 1 μl of a mixture of 100 pmoles of each of the following Dns-amino acids in 95% ethanol was applied and resolved by solvents I and II_M (A) and by solvents I and II[3,7] (B) as described in Experimental: Dns-Ala, Dns-Arg, Dns-Asp, Dns-carboxymethylcysteine (CM-Cys), Dns-cysteic acid, Dns-Glu, Dns-Gly, Dns-His, Dns-Ile, Dns-Leu, Dns-bis-Lys, Dns-ε-Lys, Dns-Met, Dns-methionine sulfone (Met-S), Dns-Phe. Dns-Pro, Dns-Ser, Dns-Thr, Dns-Trp, Dns-O-Tyr, Dns-bis-Tyr, Dns-Val and Dns-NH_2. The origin is designated by X.

In the latter method the resolution between Dns-Ile and Dns-Leu, and between Dns-Pro and Dns-Val, decreased. Solvent II_M did not improve the separation between Dns-Arg and Dns-α- or ε-Lys and another solvent must be used[3]. Typical tryptic peptides contain a C-terminal residue of either Arg or Lys. Therefore, when the peptide composition is known, there is no need to resolve Dns-Arg from Dns-Lys.

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