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Note

Identification of amino acids in picomole amounts as their 5-dibutylaminonaphthalene-1-sulphonyl derivatives

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End-group determination of peptides and proteins by dansylation is now a well-established and frequently used method[1–3]. Recently we suggested the substitution of the dimethylamino group of 5-dimethylaminonaphthalene-1-sulphonyl chloride (DANS-Cl) by the di-*n*-butylamino residue[4]. The new reagent, 5-di-*n*-butylaminonaphthalene-1-sulphonyl chloride (BANS-Cl) has some advantages in comparison with DANS-Cl. Its derivatives are less polar than the DANS-derivatives, which facilitates the isolation of the derivatives of polar compounds. BANS-derivatives have somewhat higher fluorescence quantum yields than the corresponding DANS-derivatives, and the higher yields of fragments produced by electron impact increase the detection sensitivity of BANS-derivatives by mass spectrometry. Also, the higher molecular weight facilitates their quantitative determination by mass spectrometry[5] since the background in the mass range typical for BANS-derivatives is generally very low.

Approximately ten years ago we described the first thin-layer chromatographic systems for the separation of DANS-amino acid derivatives[6], systems that subsequently proved useful in end-group determination. In the present paper we describe a simple and rapid chromatographic system that allows the complete separation of a mixture of more than 30 amino acids in picomole amounts.

MATERIALS AND METHODS

L-Amino acids were purchased from Serva (Heidelberg, G.F.R.). Usual laboratory chemicals were from E. Merck (Darmstadt, G.F.R.). BANS-Cl was prepared analogously to DANS-Cl from the corresponding sulphonic acid by reaction with phosphorus pentachloride[3,4].

Reaction of amino acids with BANS-Cl can be carried out according to the same procedure as for DANS-Cl, *i.e.*, in water–acetone (1:3) mixtures saturated with sodium carbonate[3]. Reaction volumes may vary between several microlitres and 4 ml. After completion of the reaction, either by gently shaking overnight at room temperature or by sonification of the sample vessels in an ultrasonic equipment designed for cleaning purposes for 2 h (or any other conditions suitable for the reaction of amino acids with DANS-Cl), *n*-heptane is added to the reaction mixture in an amount approximately twice the reaction volume, in order to remove the excess of reagent to-

gether with part of the side-reaction products (BANS-di-*n*-butylamide, BANS-*n*-butylamide, BANS-NH_2). After removal of the heptane phase, the water phase is neutralized by addition of solid sodium dihydrogen phosphate. The BANS-amino acids are extracted with two volumes of ethyl acetate. After appropriate concentration by a stream of air or nitrogen, this extract can be applied to the thin-layer plate.

Commercial TLC ready plastic sheets F 1700 (micropolyamide) (Schleicher & Schüll, Dassel, G.F.R.) were cut into four 7.5 × 7.5 cm pieces. Lines were scraped out of the layer 10 mm from two edges (see Fig. 1) so that reference samples could be run in the small areas thus marked off.

Development of the chromatograms was achieved by ascending chromatography in the usual solvent vapour-saturated tanks. Solvents: 1st dimension, toluene–acetic acid (9:1): 2nd dimension, water–formic acid (3:1). The solvent mixtures can be used for many runs. They are normally prepared only once during a working day.

RESULTS AND DISCUSSION

Fig. 1 shows the spot chart obtained of marker mixtures of BANS-amino acids. All separations of amino acids, essential for the chromatographic identification of end groups of proteins and peptides, may be achieved with this chromatographic system, but since the polyamide thin layers are easily overloaded, the method is only suited for the detection of picomole amounts.

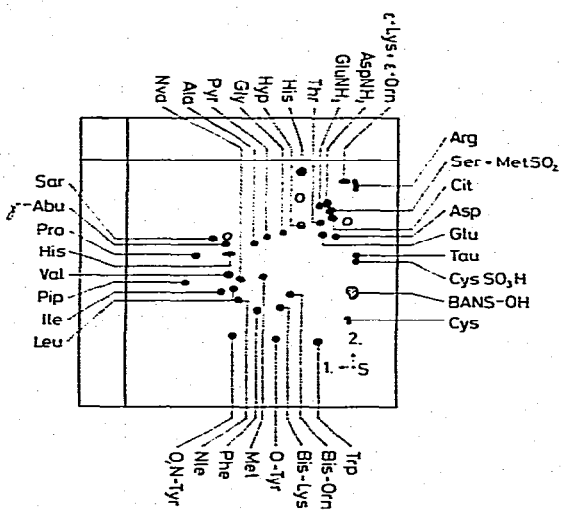

Fig. 1. Two-dimensional separation of picomole amounts of BANS-amino acids on 7.5 × 7.5 cm micropolyamide sheet. Solvents: 1st dimension, toluene–acetic acid (9:1): 2nd dimension, water–formic acid (3:1).

The usual α-amino acids appear as yellow-green fluorescing spots under a UV light source (360 nm) after the solvent has evaporated. The derivatives of histidine and pyrrolidonecarboxylic acid, and O,N-bis- and O-BANS-tyrosine appear as deep orange fluorescing spots. BANS-pyrrolidonecarboxylic acid is formed from glutamic

acid with excess reagent, analogously to the formation of the corresponding DANS-derivative[7]. BANS-2-oxopyrrolidine, which is analogously obtained by the reaction of γ-aminobutyric acid[8,9], does not appear on the chromatogram since it is extracted with heptane.

For the preparation of mass spectra of BANS-amino acids, it is necessary in certain cases for unequivocal identification[10] to wash the polyamide sheets with methanol–acetic acid (3:1) before use since the spectra exhibit otherwise high backgrounds. Extraction of the separated compounds is achieved with 30–50 μl methanol–acetic acid (3:1) using a previously described method[11].

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