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Note

Separation of 4-N,N-dimethylaminoazobenzene-4'-thiohydantoins of amino acids by thin-layer chromatography on silica gel

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We have developed a sensitive coloured reagent 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate (DABITC) for the sequence determination of peptide and proteins^{1,2}. The identification of the released N-terminal amino acids as 4-N,N-dimethylaminoazobenzene-4'-thiohydantoins (DABTH-AAs) on polyamide sheets (2.5 × 2.5 cm) is sensitive and efficient and has so far been the preferred method for the identification of DABTH-AAs. Two shortcomings in the chromatography of DABTH-AAs on polyamide have become apparent; DABTH-Leu and DABTH-Ile remain unresolved; and recovery of DABTH-AAs from the polyamide sheets is difficult unless a strong acidic solvent [such as 6 N HCl-ethanol (1:2)] is used to dissolve both DABTH-AAs and the polyamide simultaneously³.

We report here the use of silica gel for the separation of DABTH-AAs. A satisfactory resolution of DABTH-Leu and DABTH-Ile is obtained. The recovery and quantitation of DABTH-AAs could be achieved by extracting DABTH-AAs from the silica gel by ethanol or methanol.

EXPERIMENTAL

DABTH-AAs were prepared by the reaction of DABITC with excess of the amino acid in alkaline solution according to our previous report^{1,4}. The final product was separated from unreacted amino acid by extraction into ethyl acetate.

Less than 0.2 nmole of each DABTH-AA was applied to a 10 × 10 cm pre-coated plate of silica gel (Merck, G60 without fluorescent indicator, 0.25 mm) with the diameters of the applied spots confined within 1.5 mm. The plate was developed by the solvents indicated in Fig. 1. DABTH-AAs can be identified directly as yellow spots, however, greater sensitivity can be obtained by spraying the dried plate with 6 N HCl-methanol (1:30) whereby the derivatives are obtained as intense red spots against a white background.

RESULTS AND DISCUSSION

Fig. 1 shows the one-dimensional separation of DABTH-AAs on silica gel plates developed by chloroform-methanol or ethanol mixed solvents. The solvent

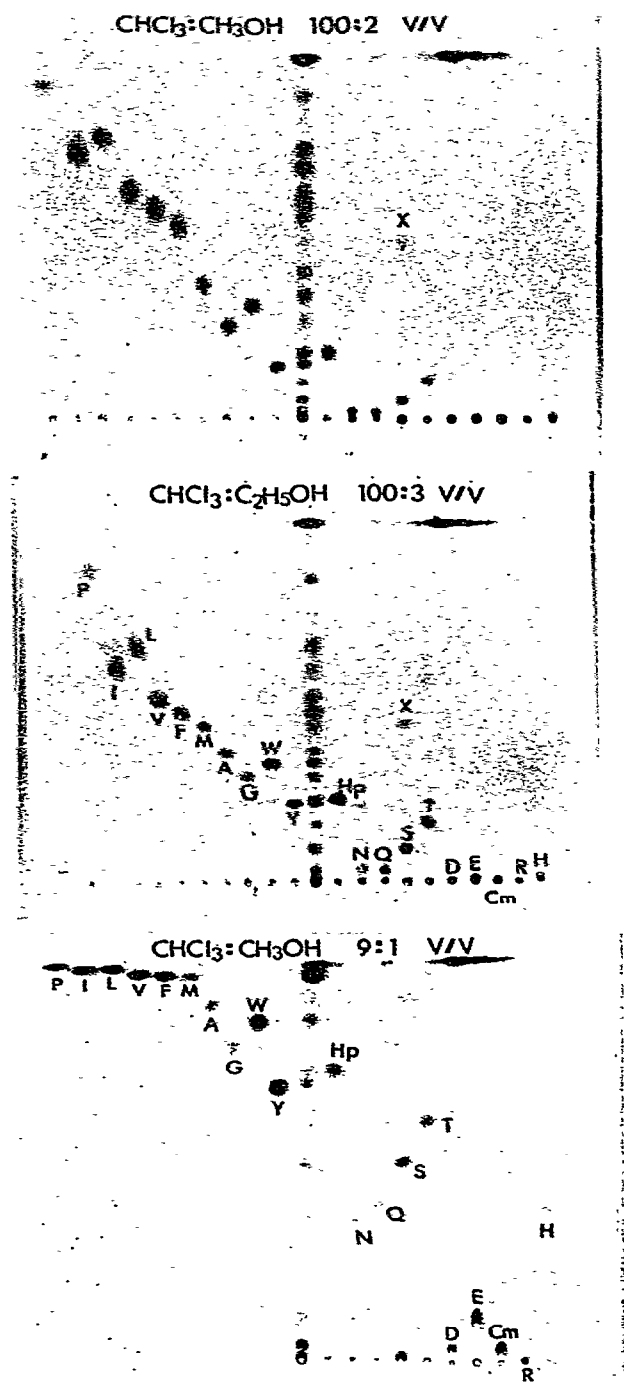


Fig. 1. Photographs of one-dimensional separation of DABTH-AAs on silica gel plate developed by the indicated solvent systems. Approximately 100 pmole of each DABTH-AA was applied. The DABTH-AAs appeared on the plates as red coloured spots after exposure to HCl vapour. Abbreviations: P = proline; I = isoleucine; L = leucine; V = valine; F = phenylalanine; M = methionine; A = alanine; G = glycine; W = tryptophan; Y = tyrosine; Hp = hydroxyproline; N = asparagine; Q = glutamine; S = serine; T = threonine; D = aspartic acid; E = glutamic acid; Cm = Carboxymethylcysteine; R = arginine; H = histidine; × = unidentified by-product of DABTH-AA (see Fig. 2)

chloroform-methanol (9:1) is suitable for separation of DABTH-Asn, DABTH-Gln, DABTH-Ser, DABTH-Thr, DABTH-Asp and DABTH-Glu. The system chloroform-ethanol (100:3) is used to separate the remaining commonly occurring DABTH-AAAs except DABTH-Asp and DABTH-CmCys which cannot be distinguished in either solvent. The R_F value of DABTH-Arg is zero in both solvents. The major by-product (\times) which is usually found during the formation of DABTH-Ser by the reaction of DABITC with serine, serylalanine and triserine can be identified in the system, chloroform-methanol (100:2). The relative position of this by-product on the polyamide sheet is shown in Fig. 2. The R_F value of α -DABTH-(ϵ -DABTC)-lysine (Bis-Lys) is 0.27 (*cf.* glycine = 0.29) in chloroform-ethanol (100:3) and 0.91 (*cf.* alanine = 0.89) in chloroform-methanol (9:1).

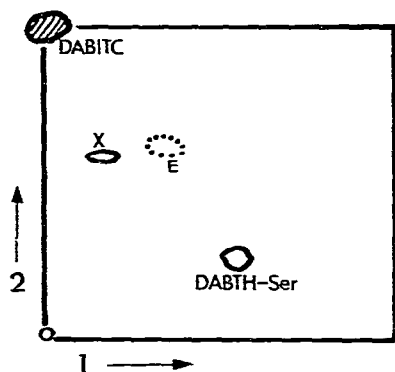


Fig. 2. Position of \times , an unidentified by-product during the formation of DABTH-Ser, on a two-dimensional polyamide sheet developed with water-acetic acid (2:1) (solvent 1) and toluene-*n*-hexane-acetic acid (2:1:1) (solvent 2). This by-product is believed to possess a thiohydantoin structure since it appeared as a red spot after exposure to HCl vapour. E is the authentic blue marker 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl diethylamine.

Cheng Chin (Taipei, Taiwan) polyamide sheets have provided one of the most elegant mediums for the qualitative detection of amino acid derivatives^{1,5-8}. The sturdy structure of the absorbent, however, makes the recovery of DABTH-AAAs tedious. For quantitation using spectrophotometrical measurement, we have used a strongly acidic solvent, 6 *N* HCl-ethanol (1:2) to dissolve both DABTH-AAAs and the polyamide absorbent simultaneously³. This procedure however could possibly cause the destruction of some acid labile DABTH-AAAs. The recovery of DABTH-AAAs from silica gel is relatively simple. A number of solvents, such as ethanol, methanol or acetone, can be used to extract DABTH-AAAs, the silica being easily removed by centrifugation.

Perhaps the most significant result of DABTH-AAAs separation on silica gel plates is the resolution of DABTH-Leu and DABTH-Ile which we have failed to achieve by using polyamide sheets. The silica gel plates, therefore, should be used in combination with polyamide sheets whenever discrimination between leucine and isoleucine or quantitation of DABTH-AAAs is needed.

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