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Note

Separation of 4-N,N-dimethylaminoazobenzene-4′-sulfonyl amino acids on polyamide sheets

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4-N,N-Dimethylaminoazobenzene-4′-sulfonyl chloride (DABS-Cl) is a sensitive chromophoric azo compound synthesized from the reaction of methyl orange and PCl_5[1]. The sulfonyl group of DABS-Cl readily reacts with primary and secondary amino groups, thiols, imidazoles, phenols and aliphatic hydroxyl groups. The effectiveness of DABS-Cl in the qualitative and quantitative identification of amino acids (AAs) and in the determination of the N-terminal amino acids of peptides and proteins using silica gel plates, and the investigation of the physical properties of seventeen DABS-AAs have been reported[1]. The intense chromophoric DABS-AAs permit the detection of amino acids as colored spots in the range of 10^{-10} to 10^{-11} moles visible directly on the thin-layer plate. But due to the diffusion problem on silica gel plates which decreases the sensitivity and causes unsatisfactory separation of certain DABS-AAs, such as Leu, Ile and Val, Met and Phe, Ala and Pro, we endeavoured to find a better separation of DABS-AAs and in this report investigated polyamide sheets for the purpose.

EXPERIMENTAL

Cheng Chin polyamide layer sheets were purchased from Pierce and were cut into 5 × 5-cm squares prior to use. All solvents used were commercial analytical grade without further purification.

50 nmoles of each amino acid dissolved in 50 μl of 0.2 M $NaHCO_3$ was allowed to mix with 50 nmoles of DABS-Cl in 50 μl acetone and reacted at 70° for 5–10 min. About 10–15 picomoles of each DABS-AA was successively applied to the origin of the plate. In order to obtain a better separation, the diameter of the original spot should be confined to 1.0–1.3 mm by using a mild hair dryer.

Two-dimensional development in a covered jar was performed by ascending solvent flow[2,3]: solvent 1, water–2-chloroethanol–formic acid (100:60:3.5, v/v/v) for the first dimension development and solvent 2, benzene–acetic acid (6:1, v/v) for the second dimension.

RESULTS AND DISCUSSION

Fig. 1 shows the original developed plate after being exposed to HCl vapor.

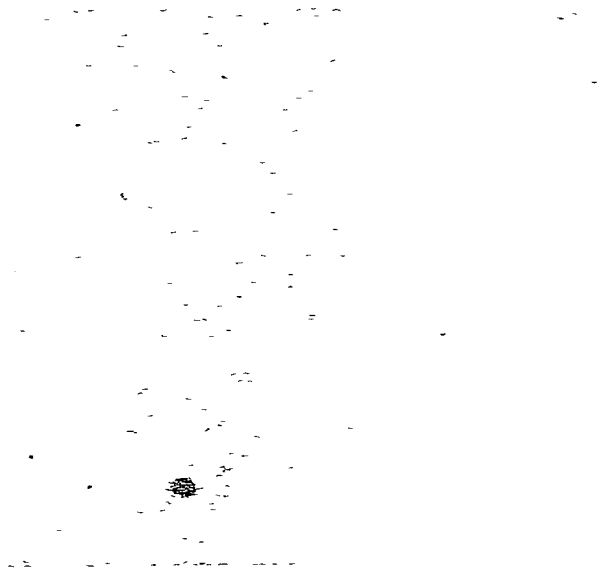


Fig. 1. Photograph of a two-dimensional development 10–15 picomoles of each DABS-AA was applied. The spots appeared on the sheet as red colored after exposure to HCl vapor.

It shows that the separation between many DABS-AAs was quite satisfactory. Fig. 2 is a schematic representation of 30 different DABS-AAs on a two-dimensional plate. However, only 23 individual spots appeared on the plate, the other 7 DABS-AAs were incompletely separated and give four spots, *i.e.*, $CysO_3H$ and DABSOH; Hyp,

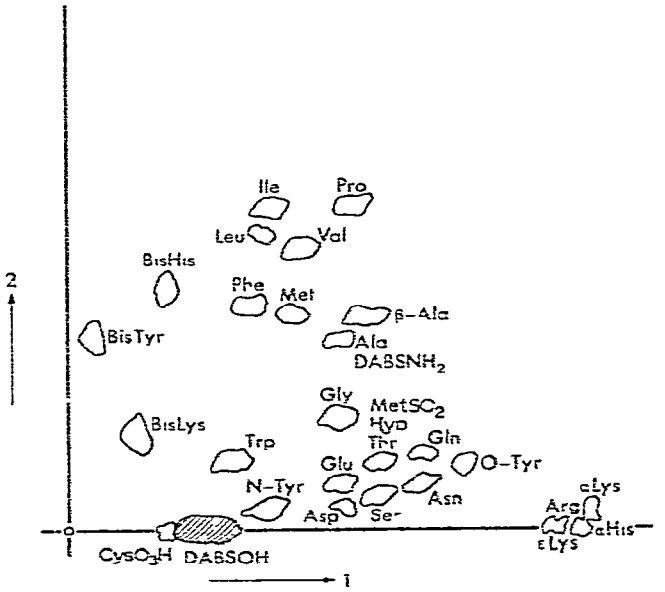
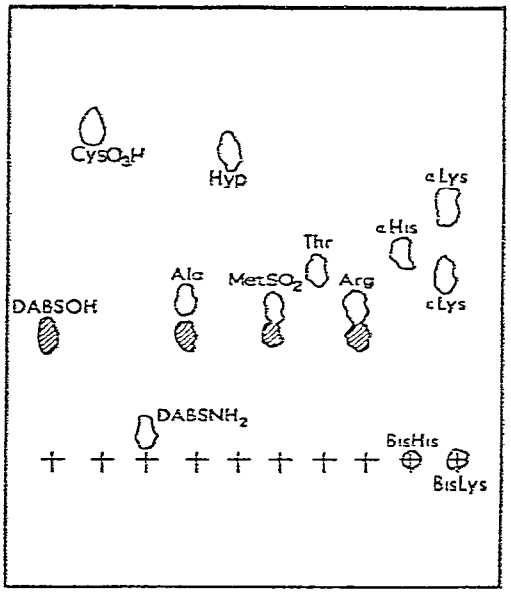


Fig 2. A schematic representation of the two-dimensional chromatography of 30 DABS-AAs. Details are given in the text.



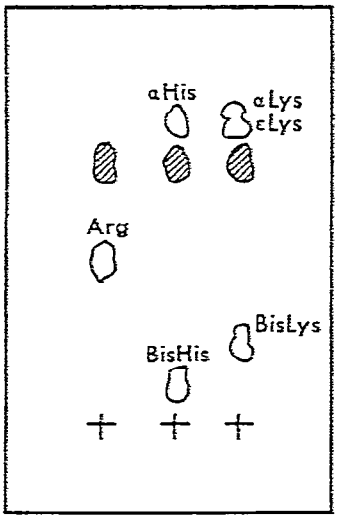


Fig. 3 One-dimensional separation of four overlaps: DABSOH and $CysO_3H$, $DABSNH_2$ and Ala, $MetSO_2$, Hyp and Thr; α-monoHis, α-monoLys, ε-monoLys and Arg by solvent 4. Details. see text

Fig. 4. One-dimensional chromatography using solvent 3 (see text) for the identification of Arg from mono-substituted Lys and His.

$MetSO_2$ and Thr: Ala and $DABSNH_2$; α-monoHis, α-monoLys, ε-monoLys and Arg. Solvent 4, water–pyridine–ammonia (28%)–formic acid (100:20:10:2), was found to be extremely satisfactory for resolving these four overlaps and solvent 3, water–ammonia (28%)–ethanol (9:1:10), for identification of arginine (Figs. 3 and 4). The identification of the mono-substituted bifunctional amino acids tyrosine and lysine was carried out by dabsylation and hydrolysis of carbobenzoxy, benzyl and acetyl protected amino acids.

α-Monohistidine was confirmed by hydrolyzing BisHis in 6 *N* HCl for 2 h. The diffusion problem on polyamide has been proved to be much less than that on silica gel and hence increases the sensitivity of detection on polyamide plate. The diameters of the separated spots were limited to approximately 2 mm when the diameter of the original spot was confined to 1.0–1.3 mm. In addition, the whiter background of polyamide sheet has facilitated the observation of the separated spots. Under these circumstances, we have been able to detect the spots of DABS-AAs up to picomole level without any need for an ultraviolet lamp or chemical indicators. Furthermore, this sensitive colorimetric method was expected to offer a much more convenient procedure in quantitative analysis and column chromatographic separation of DABS derivatives.

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