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Note

Paper chromatographic behaviour of α-DNP-lysine in comparison with other DNP-amino acids

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It has been demonstrated that L-lysine serves as a branching point of the polypeptide chain, in materials such as biocitin[1], bacitracin[2] and the bacterial cell wall[3], the ε-amino group of the L-lysine participating in the formation of an amide bond. It would be expected that α-DNP-lysine would be obtained from biocitin, in which the ε-amino group is bound with a biocityl group and the α-amino group is free, by dinitrophenylation followed by hydrolysis. However, no report on the clear separation of α-DNP-lysine from other DNP-amino acids could be found, and we have therefore examined this separation by one- and two-dimensional paper chromatography.

EXPERIMENTAL

DNP-amino acids

α-DNP-lysine was synthesized by the methods of Bezas and Zervas[4] and Sanger[5]. The product was characterized as α-DNP-lysine by various physico-chemical measurements: NMR and IR spectroscopy, m.p. determination and elemental analysis. Other DNP-amino acids were purchased from Seikagaku Kogyo (Tokyo, Japan).

Paper chromatography

The paper chromatographic separation of DNP-amino acids was performed by ascending development on Toyo No. 51 filter-paper with the solvent systems (1) pyridine–isoamyl alcohol–1.6 *M* ammonia solution (3:7:10)[6], (2) *tert.*-amyl alcohol–0.1 *M* phthalate (pH 6)[7] and (3) 1.5 *M* sodium phosphate (pH 6)[8]. A 2% solution of ninhydrin in *n*-butanol and Sakaguchi reagent[9] were used for staining the chromatograms.

RESULTS AND DISCUSSION

The one-dimensional paper chromatographic separation of α-DNP-lysine is shown in Fig. 1. α-DNP-lysine was separated clearly from ε-DNP-lysine using the above solvent systems. Solvent system 2 was useful for the separation of water-

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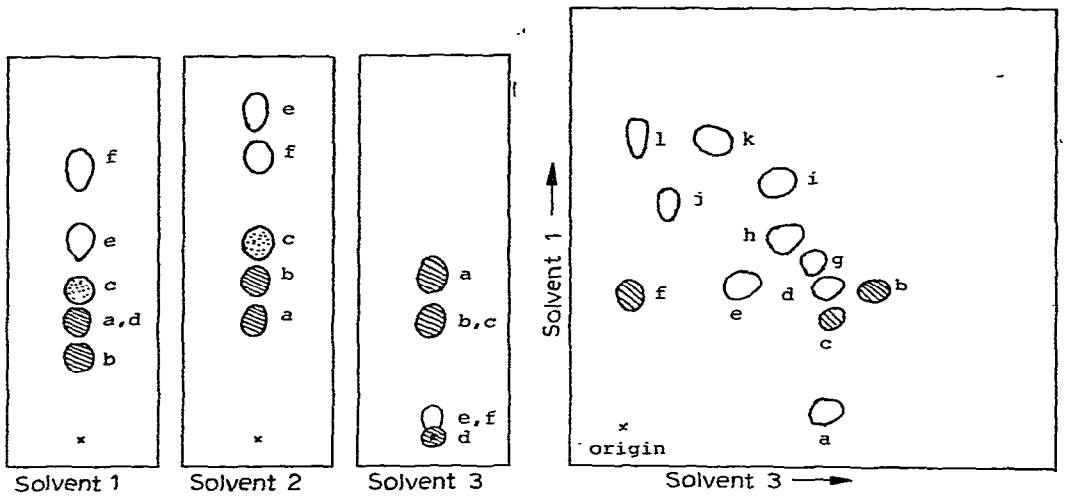

Fig. 1. Paper chromatographic separation of α-DNP-lysine from ε-DNP-lysine and α,ε-di-DNP-lysine. Solvent systems: see text. Spots: a, α-DNP-lysine; b, ε-DNP-lysine; c, α-DNP-arginine; d, *o*-DNP-tyrosine; e, di-DNP-histidine; f, di-DNP-lysine. Hatched spots are the ninhydrin-positive spots and the dotted spots are the Sakaguchi-positive spots.

Fig. 2. Two-dimensional paper chromatographic separation of α-DNP-lysine from other DNP-amino acids. Solvent systems: first development, solvent 1; second development, solvent 3. Spots: a, DNP-aspartic acid; b, α-DNP-lysine; c, ε-DNP-lysine; d, DNP-serine; e, DNP-glycine; f, *o*-DNP-tyrosine; g, α-DNP-arginine; h, DNP-alanine; i, DNP-leucine; j, di-DNP-histidine; k, DNP-phenylalanine; l, di-DNP-lysine. Hatched spots are the ninhydrin-positive spots.

soluble DNP-amino acids, including α-DNP-lysine. The spot of α-DNP-lysine was coloured by ninhydrin reagent, as was that of ε-DNP-lysine. The spot of α-DNP-arginine on a paper chromatogram developed with solvent system 1 or 2 could be detected with the Sakaguchi reagent, but could not be detected on a chromatogram developed with solvent system 3.

Solvent systems 1 and 3 were useful for the two-dimensional separation of α-DNP-amino acids, as shown in Fig. 2. Solvent system 1 should be used for the first development and 3 for the second development. Both the mono-DNP-lysines and other water-soluble DNP-amino acids and the ether-soluble DNP-amino acids used in this work were clearly separated by these solvent systems. Therefore, this two-dimensional solvent system is expected to be useful for the quantitative separation of α-DNP-lysine as well as other DNP-amino acids.

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