Micropolyamide Thin-layer Chromatography of Fluorescein-thiohydantoin-Amino Acids at Picomole Level

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Fluorescein-isothiocyanate has been shown to react with the α -amino group of amino acid, peptide and protein. The reaction takes place at the N-terminal of peptide. The resulted fluorescein-thiocarbamyl-peptide degrades to fluorescein-thiohydantoin (FTH)-amino acid corresponding to the N-terminal amino acid. This is a kind of Edman's degradation.^{1,2)} FTH-amino acids can be chromatographed by thin-layer silica gel³⁾ and are detectable by the intense greenish-yellow fluorescence under ultraviolet light. In this paper we report on a rapid and sensitive TLC-technique using a micropolyamide sheet. Most protein constructing amino acids except FTH-Leu and FTH-Ile, were successfully separated.

Applications of micropolyamide TLC-technique for phenylthiohydantoin-amino acids⁴⁾ and 1-dimethylamino-naphthalene-5-sulfonyl amino acids⁵⁾ have been known.

Double faced micropolyamide sheets (Cheng Ching Trading Co., Ltd., Taiwan) were obtained from Seikagaku Kogyo Co., Ltd. (Tokyo). The commercial sheet (15×15 cm) was cut into nine pieces (5×5 cm each). The preparation of FTH-amino acids has been previously described.³⁾ All solvents were purified to meet chromatographical requirements.

Application of FTH-amino acids onto the sheet and the ascending chromatography were carried out in accordance with Kulbe's method on phenylthiohydantoin-amino acids.⁴⁾

The following solvent systems were selected; (I) acetone: benzene: glacial acetic acid (4: 2: 1, by vol.); (II) water: pyridine: glacial acetic acid (10: 8: 1, by

Fig. 1. Two-dimensional Thin-layer Chromatography of FTH-amino Acids.

Conditions are described in the text. Spot no. are identified in Table I.

TABLE I. *R_f* VALUES OF FTH-AMINO ACIDS ON THIN-LAYER OF POLYAMIDE

The composition of the solvent systems and the experimental conditions are described in the text.
FITC-I, fluorescein-isothiocyanate isomer I; AF-I, aminofluorescein isomer I.

FTH-amino acids	Solvent system			FTH-amino acids	Solvent system		
	I	II	III		I	II	III
(1) Gly	0.60	0.63	0.42	(12) Trp	0.57	0.45	0.42
(2) Ala	0.65	0.60	0.57	(13) Tyr	0.41	0.51	0.40
(3) Val	0.70	0.49	0.70	(14) Asn	0.50	0.69	0.21
(4) Leu	0.73	0.50	0.75	(15) Asp	0.24	0.80	0.08
(5) Ile	0.72	0.49	0.73	(16) Gln	0.61	0.68	0.25
(6) Phe	0.69	0.52	0.65	(17) Glu	0.40	0.69	0.21
(7) Pro	0.79	0.63	0.70	(18) Lys	0.06	0.11	0.03
(8) Ser	0.43	0.63	0.42	(19) Arg	0.31	0.77	0.01
(9) Thr	0.52	0.60	0.55	(20) His	0.51	0.76	0.39
(10) CMCys	0.10	0.69	0.06	(21) FITC-I	0.65	0.55	0.71
(11) Met	0.68	0.51	0.50	(22) AF-I	0.54	0.74	0.63

vol.); and (III) hexane: methanol: pyridine (10: 3: 2, by vol.). Most FTH-amino acids were separated with the above solvents at levels comparable to those on silica gel plate. R_f values of FTH-amino acids on polyamide sheet are shown in Table I. A two-dimensional chromatogram using solvent I followed by solvent II is illustrated in Fig. 1. No solvent system has been found to separate FTH-Leu and FTH-Ile. R_f values of FTH-Val and FTH-Phe were close on solvent I. The separation was not clear even by two-dimensional chromatography. The solvent III, however, gave relatively good resolution for these derivatives. Care must be taken in the case of sequence analysis. In Fig. 1, FTH-carboxymethyl-cysteine (No. 10) was accompanied by three by-products (No. 10'). Similarly FTH-Lys showed two spots, which are probably mono-labeled derivatives at α - and ϵ -amino group.

The sheet size 5×5 cm was sufficient for separation and economical. The R_f value of FTH-amino acid was very much affected by vaporization of the developing solvent. Care must be taken to saturate enough the developing jar with the solvent vapor. The spot containing more than 10^{-9} mole of FTH-amino acid was detectable by yellowish color. The spot containing 10^{-12} mole was detectable by intense greenish-yellow

fluorescence under ultraviolet light. More sensitive identification was possible by exposing the chromatogram to ammonia gas to bring it to an alkaline state after all solvent was evaporated.

FITC is a new type of Edman reagent, in which fluorescein chromophore is attached instead of the phenyl group. The detection limit of FTH-amino acids is 10^{-12} mole on TLC. This derivative is more sensitive than PTH-amino acid which is detectable by the fluorescent indicator in the range of $0.5 \sim 2 \times 10^{-10}$ mole.⁴⁾

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