RAPID THIN LAYER CHROMATOGRAPHIC SEPARATION OF PARA-PHENYLAZOPHENYL THIOHYDANTOINS OF AMINO ACIDS

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SUMMARY

3 (p-phenylazophenyl) 2-thiohydantoins of amino acids were identified on silica gel plates in 30 mins using ascending thin layer chromatographic technique. Recovery percentages of these amino acid derivatives were calculated and observed that the method of preparation needed modification.

INTRODUCTION

In our previous communications (1,2) we reported that p-phenylazophenyl isothiocyanate (PAPITC) could be conveniently used to determine N-terminal amino acid sequences of proteins. When the reagent was treated with a protein, colored 3 (p-phenylazophenyl) 2-thiohydantoin (PAPTH) of amino acids were degraded from the N-terminal end stepwisely. Due to intense color, visual identification of these amino acid derivatives were possible through thin layer chromatography (TLC).

Previously we used three solvent systems (1) to separate amino acid PAPTHs and in each case at least $2\frac{1}{2}$ hrs were required to separate these amino acid derivatives.

Identification of degraded amino acid PAPTHs from proteins through a rapid method is most essential when amino acid sequence determination of a protein is carried out automatically. This is also true in case of routine analysis when carried out mannually. In

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the present work, we have conducted ascending TLC to identify PAPTHs of 21 amino acids in 30 mins only.

MATERIALS AND METHODS

Preparation of amino acid PAPTHs :

Procedure of Datta, Datta and Sengupta (1) was used to prepare amino acid PAPTHs after some modification which are as follows. In the first step of reaction 1 milimole pure amino acid was dissolved in 1 ml water and pH was adjusted to 11.8 instead of 9.7 by adding 2N NaOH instead of N/10 alkali. 0.048 gms of PAPITC was dissolved in 2 ml dioxan and mixed with the solution of amino acid. Further alkali was added to maintain the pH at 11.8.

Ascending TLC of amino acid PAPTHs :

TLC plates made up of ground glass (20 x 10 cm) were spread with a 0.4 mm thick layer of silica gel slurred in absolute alcohol and dried overnight at room temperature. Amino acid PAPTHs were taken in acetone and spotted on these plates and air-dried at room temperature. Spots contained 50-60 μg samples. The plates were then placed in the developing chambers previously saturated with the following solvent systems.

Solvent I

Acetonitrile : Ammonium acetate (0.2 M) : Acetone (75:15:10)

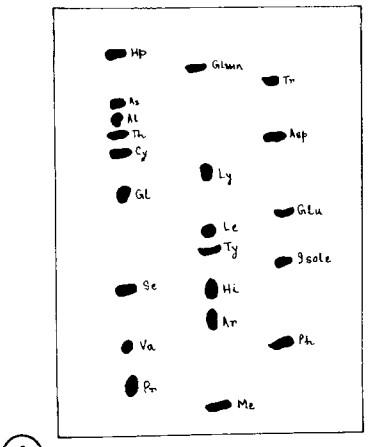
Solvent II

Acetonitrile : Ammonium acetate (0.1 M) : Acetone (55:35:10)

Spots were allowed to develope for 30 mins only.

Quantitative estimation of amino acid PAPTHs :

Separated amino acid PAPTHs were carefully scraped out and taken with 4 ml ethyl acetate in a centrifuge tube. After stirring for 15-20 mins it was centrifuged and the centrifugate was read at 535 nm in a DBG-Beckman spectrophotometer. Amounts of amino acid PAPTHs were calculated from a standard graph. Arginine and histidine PAPTHs were taken with methanol instead of ethyl acetate (1).



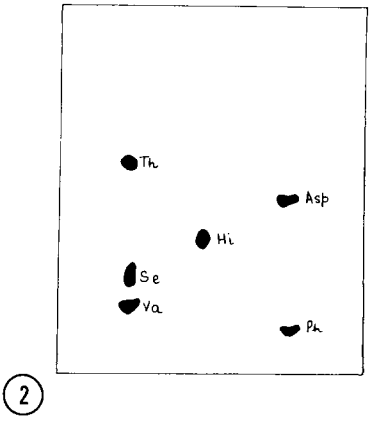


Fig. 1. Separation of amino acid PAPTHs by ascending TLC. Hydroxyproline (Hp), Asparagine (As), Alanine (Al), Cystine (Cy), Glycine (Gl), Serine (Se), Valine (Va), Proline (Pr), Glutamine (Glmn), Lysine (Ly), Leucine (Le), Tyrosine (Ty), Histidine (Hi), Arginine (Ar), Methionine (Me), Tryptophan (Tr), Aspartic acid (Asp), Glutamine (Glu), Isoleucine (Isole), Phenylalamine (Ph), Threonine (Th)

Solvent system

Acetonitrile:Ammonium acetate (0.2M):Acetone (75:15:10)

Fig. 2. Separation of Threonine (Th), Aspartic acid (Asp), Serine (Se), Histidine (Hi), Valine (Va) and Phenylalanine (Ph) PAPTHs by ascending TLC

Solvent system

Acetonitrile:Ammonium acetate (0.1M):Acetone (55:35:10)

Recovery experiment :

21 synthetic amino acids (10 μ mole each) were mixed up and the reaction with PAPITC was carried out according to the present method and Datta, Datta and Sengupta's method (1) separately. In each case 15 μ mole of the reacted mixture was applied to the TLC plates and developed with the present solvent system.

Table I

Recovery of Amino acid PAPTHs

Amino acid PAPTH	Rec. (/)	
	Method of Datta, Datta, and Sengupta (1)	Present method
dl-Glycine	83.1	97.4
dl-Alanine	85.4	98.5
l-Threonine	80.6	91.2
l-Proline	79.3	94.5
l-Tyrosine	87.2	95.6
dl-Valine	77.3	90.2
l-Glutamine	88.2	96.0
dl-Serine	73.4	91.9
l-Leucine	78.4	95.2
l-Isoleucine	91.4	98.6
dl-Aspartic acid	81.9	93.9
l-Hydroxyproline	71.7	92.6
l-Asparagine	85.0	89.8
l-Glutamic acid	89.8	99.2
l-Phenylalanine	93.5	98.0
dl-Methionine	80.3	95.5
l-Cystine	76.5	93.7
l-Lysine	68.6	82.2
l-Tryptophan	69.1	80.2
l-Arginine	77.4	84.6
l-Histidine	61.8	79.2

RESULTS AND DISCUSSION

In Fig. I separation of amino acid PAPTHs has been cited. It will be observed that except Threonine, Aspartic acid, Serine,

Histidine, Valine and Phenylalanine PAPTHs all other amino acid derivatives resolved well in 30 mins using the first solvent system (solvent I). PAPTH-leucine and PAPTH-isoleucine which are generally very difficult to separate, were also resolved in this solvent system.

To separate PAPTHs of Threonine from Aspartic acid, Serine from Histidine and Valine from Phenylalanine a second solvent system (solvent II) was used. The thin layer chromatogram is furnished in Fig. 2. It is clear that the aforesaid amino acid PAPTHs were also perfectly resolved in 30 mins.

Modification of the method of Datta, Datta and Sengupta (1) was necessary because from table I it is revealed that the recovery percentage of amino acid PAPTHs when prepared according to the method of Datta, Datta and Sengupta (1) were lower than those prepared by the present method. This proves that modification of the previous method (1) was necessary.

We dissolved amino acids and PAPITC separately in water and dioxan respectively because we observed that minimum time was required when the reaction was carried out by taking these two components in solution. pH was adjusted at 11.8 instead of 9.7 because we observed that in previous procedure (1) certain amount of PAPITC was left unreacted at pH 9.7 but at pH 11.8 this does not occur and the color of the solution becomes deeper.

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REFERENCES

1. Datta, S., Datta, S.C., and Sengupta, R. (1976) Biochem. Biophys. Res. Commun. 72, 1296-1303.

2. Datta, S., Datta, S.C., and Sengupta, R. (1977) J. Dairy Sci. (in press).