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NOTE

Separation and Polychromatic Identification of Some Amino Acids Present in Carrot Protein

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Abstract

An efficient one dimensional thin-layer chromatography separation of seven amino acids known to be present in carrot was developed. The thin-layer plates were coated with an adsorbent of microcrystalline cellulose. Several solvent systems were evaluated with a butanol, acetone, water, dicyclohexane solvent giving a good separation of the amino acids. Detection was accomplished by spraying with ninhydrin.

INTRODUCTION

The use of thin-layer chromatography (TLC) in the identification of amino acids as well as their separation has grown to great importance since this technique was first used by Martin on paper (*1*). While silica gel has been the main adsorbent for TLC, we have recently shown that microcrystalline cellulose can be successfully used as an adsorbent in inorganic TLC (*2–5*). This application includes the separation of a number of inorganic cations, anions, metal–EDTA complexes, and transition metal complexes.

Two-dimensional TLC on silica gel was used to separate amino acids from hot water extracts of carrot (*6*). The method reported here uses a one-dimensional TLC technique to separate and identify some of the ami-

no acids present in carrot protein, using microcrystalline cellulose as the adsorbent.

EXPERIMENTAL

Plate Preparation

A rather fluid slurry of the adsorbent was prepared by blending 50 g of microcrystalline cellulose (technical grade, FMC Corp., Marcus Hook, Pennsylvania) with 218 ml of distilled water in a Waring blender for 10–15 sec at low speed and a few seconds at high speed. Air bubbles were removed from the slurry by gentle shaking and pumping with a water aspirator for 2 min. The mixture was spread on 20 × 5 cm glass plates at a thickness of 0.75 mm. After setting for about 5 min the plates were separated by use of a spatula and allowed to dry overnight.

Carrot Preparation

Slices of carrots (approx 1.5 g) were pulverized in acetone in a Waring blender for 3 min at low speed. The acetone was removed and the carrot material was repulverized in acetone for an additional 2 min to remove any excess pigment. The resulting plant material was vacuum filtered and dried on the funnel for 15 min. Approximately 1 g of a fluffy white product was transfered to a 250-ml round-bottomed flask to which was added 100 ml of 6 *N* HCl. The material was refluxed on an oil bath at 140–145°C for 23 hr (*7*). After hydrolysis the carrot material changed from a brown to black color, probably due to the decomposition of sugars. On vacuum filtering the resulting solution through a fritted glass funnel, approximately 100 ml of an orange hydrolyzate was obtained with a black residue on the funnel.

Chromatography

A solution suitable for chromatographic separation was prepared by passage of 10 ml of the product solution through a Dowex 50W × 8 column at approximately 1 ml/3 min and washing the column with about 12 ml of deionized water at the same rate (*8*). The chromatographic solution was obtained by eluting the column with 30 ml of 17% NH_3 followed by vaccum evaporation and taking up the yellow orange residue in 0.2 ml of 0.5 *N* HCl.

Table 1 lists the various solvent systems used. These are variations of previously reported systems used on paper and TLC (*9*, *10*). Previous to

TABLE 1

Solvent Systems Tested for Chromatographic Separation

System	Composition by volume
A	1-Butanol: acetic acid: water (4: 1: 5)
B	Chloroform: methanol: ammonia (17%) (2: 2: 1)
C	Ethanol: 1-butanol: water: cyclohexylamine (10: 10: 5: 2)
D	Ethanol: 1-butanol: water: dicyclohexylamine (10: 10: 5: 2)
E	1-Butanol: acetone: water: dicyclohexylamine (10: 10: 5: 2)
F	1-Butanol: acetone: water: cyclohexylamine (10: 10: 5: 2)

testing the solvent on microcrystalline cellulose, preliminary tests of the solvents were run by circular chromatography using 7 cm Whatman (No. 1) filter circles. This procedure involved the making of a hole in the center of the circle (6–8 mm in diameter) and inserting into the hole a rolled strip of chromatography paper (1 × 3 cm). Samples were spotted 3 mm from the edge of the hole. The chromatograms were developed in plastic Petri dishes with enough solvent to cover the bottom of the dish. After equilibration (15 min) of the solvent, with the top cover on, the filter circle was lowered into the bottom dish using a "Bic" pen cover inserted into the circle hole of the paper cylinder. Careful removal of the pen cover allowed the filter circle to stand on the paper cylinder immersed in the solvent system. This enclosed system provided a quick and easy evaluation of the solvent system, taking 10–15 min of developing per chromatogram in the various solvent systems.

The TLC separations were done in chambers containing 100 ml of chromatographic solvent. The developing tanks were 10 × 10 × 3 in. (such as the ones available from Brinkman Instruments Inc., Westbury, New York). The development time varied with each solvent system: 150 min for A, 60 min for B, 100 min each for C and D, and 80 min each for E and F.

Visualization

The visualization reagent consisted of a mixture of 50 ml of 0.2% (w/v) ninhydrin in absolute ethanol, 10 ml of acetic acid, and 2 ml of collidine. A second solution consisted of 1% (w/v) $Cu(NO_3)_2 \cdot 3H_2O$ in absolute ethanol. The two solutions were mixed in a 50: 3 ratio prior to use. Detection was accomplished by evaporation of the solvent by use of a heat gun, followed by spraying of the visualization reagent onto the TLC plate and heating again for about 1 min. This procedure required 3 to

5 min for paper chromatograms and 5 to 8 min for TLC. Each amino acid gave a characteristic color with the indicator.

Amino Acid Identification

Individual solutions (50 mg of acid in 5 ml 0.5 *N* HCl) of the seven amino acids known to be present in carrot were prepared as well as one mixture of 25 mg of each acid in 5 ml of 0.5 *N* HCl (*11*). These eight solutions were chromatogramed and their separations were compared to the concentrated sample obtained from the carrot. The optimum separation came from a plate containing 8 to 10 individual applications on the same spot for the carrot sample as compared to one application of each of the known acids. The characteristic colors on detection with ninhydrin faded with time.

RESULTS AND DISCUSSION

Of the solvents tested, A, D, and E gave the most favorable separations. Solvent B was designed for silica gel and it exhibited a poor separation on microcrystalline cellulose. Solvents C and F developed dark backgrounds on heating, and visualization of the amino acids was not possible. Solvent E was chosen over A because A acted differently under slight changes of conditions, probably due to its two-phase character. Advantages which E exhibited over D include a broader range of R_F values and the time required for development of the chromatogram. Table 2 lists the R_F values of amino acids present in carrot protein as well as their characteristic color by ninhydrin in Solvent E. No significant change in R_F value was noted for the amino acids found in carrot versus the known amino acids chromatogramed individually or in the synthetic mixture of

TABLE 2

Amino Acids Found in Carrots by TLC Using Microcrystalline Cellulose

Amino acid	R_F value in Solvent E	Ninhydrin color
Valine	0.63	Purple
Serine	0.59	Yellow
Glutamic	0.53	Brown
Aspartic	0.47	Blue-violet
Glycine	0.43	Brownish red
Lysine	0.19	Reddish brown
Arginine.	0.09	Purple

amino acids. The good separation as indicated by the R_F value plus the characteristic ninhydrin color for each amino acid in Solvent E indicates the applicability of this technique. The microcrystalline cellulose TLC plates give a remarkably hard surface which stands up in a variety of solvents.

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