Chromatography of dinitrophenyl amino acids on silica gel-impregnated glass paper*

The reaction of dinitrofluorobenzene (DNFB) with amino acids, peptides and proteins was the key to the solution of the primary structure of insulin[1]. This reaction has retained a position of importance in studies of the amino acid sequence and composition of proteins and peptides[2]. The dinitrophenyl (DNP) amino acid and peptide products of the reaction have been separated by distribution between solvents, electrophoresis, column chromatography on silica gel, and by paper chromatography; however, breakdown products of DNFB or of DNP-amino acids often complicate analysis of DNP-derivatives. Preliminary solvent extraction or time-consuming treatment to remove buffers or acid contaminants is generally necessary prior to chromatography. Most methods require hours for completion. The method presented here overcomes many of these difficulties.

Methods

Ascending chromatography is performed using glass paper impregnated with silica gel. Such paper may be prepared[3] or purchased from Applied Sciences, Inc. of State College, Pa. Standard DNP-derivatives of amino acids are easily prepared[2] or can be purchased from a number of supply houses. Standards (1 to 10 mmole/l) are dissolved in methanol or water. Spots 3 to 4 mm in diameter are applied to paper with melting point capillaries. Resolution is best when less than 2 μg of a specific derivative is applied.

R_F values of DNP-derivatives in three solvent systems are presented in Table I. Although R_F values obtained in a specific solvent system may vary slightly from those recorded in Table I, the relative positions of derivatives to each other are constant. Before analyzing an unknown one should adjust the composition of the solvent system until a standard derivative migrates with the desired R_F value. Any two or more compounds listed may be separated by a single chromatographic run or by two-dimensional chromatography. In general, the first dimension is used to separate an unknown into groups, and the second to isolate specific compounds within a group. The isooctane–chloroform–acetic acid solvent system is used primarily for the group separation; the ether systems are used in the second dimension for separations of derivatives within a group.

The isooctane–chloroform–acetic acid (100:100:10) solvent system separates the derivatives into five groups. Group I. Highly polar derivatives remain near the origin. These include DNP-dicarboxylic amino acids, their amides, hydroxy DNP-amino acids, etc. Group II. DNP-glycine, DNP-tryptophane and the di-DNP-derivatives of lysine and tyrosine cluster about R_F 0.25. Group III. Proline, phenylalanine, alanine and methionine derivatives move to the region of R_F 0.5. Group IV. DNP-leucine, DNP-isoleucine and DNP-valine have R_F values of about 0.7. Group V. DNFB and breakdown products of DNFB and DNP-amino acids such as dinitrophenol and dinitroaniline move with the solvent front.

Compounds in Groups I and II are separated by chromatographing in the first dimension in ether–acetic acid–water (100:3:3) and, in the second dimension, in

* Supported by NSF Grant G-10751 and USPHS Grant AM 05967.

TABLE I

R_F VALUES OF DINITROPHENYL AMINO ACIDS IN THREE SOLVENT SYSTEMS

Compound	*Isooctane system**			*Acid ether system***			*Alkaline ether system****		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Group I									
DNP-L-Arginine	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.14	0.78
Di-DNP-L-histidine	0.00	0.00	0.00	0.00	0.04	0.07	0.11	0.38	0.94
ε-DNP-L-Lysine	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.03	0.37
Di-S,N-DNP-L-cysteine	0.00	0.02	0.06	0.16	0.49	0.83	0.40	0.96	0.95
N,N'-Di-DNP-L-cystine	0.00	0.00	0.00	0.00	0.16	0.53	0.00	0.14	0.72
DNP-DL-Methionine sulfoxide	0.00	0.00	0.00	0.00	0.14	0.36	0.02	0.28	0.76
DNP-L-Serine	0.00	0.00	0.02	0.24	0.55	0.85	0.07	0.36	0.92
DNP-L-Threonine	0.00	0.02	0.08	0.28	0.65	0.90	0.13	0.49	0.97
DNP-L-Asparagine	0.00	0.00	0.00	0.02	0.32	0.58	0.00	0.24	0.71
DNP-L-Glutamine	0.00	0.00	0.00	0.03	0.35	0.58	0.01	0.17	0.82
DNP-L-Aspartic acid	0.00	0.00	0.04	0.04	0.42	0.72	0.00	0.00	0.02
DNP-DL-Glutamic acid	0.00	0.04	0.10	0.15	0.58	0.85	0.00	0.00	0.03
Group II									
Di-N,N'-DNP-L-lysine	0.02	0.17	0.29	0.35	0.77	Front	0.26	0.92	Front
Di-O,N-DNP-L-tyrosine	0.02	0.20	0.33	0.40	0.80	Front	0.37	0.99	Front
DNP-Glycine	0.06	0.25	0.31	0.51	0.83	Front	0.16	0.58	Front
DNP-L-Tryptophane	0.09	0.27	0.40	0.79	0.97	Front	0.29	0.86	Front
Group III									
DNP-L-Alanine	0.24	0.50	0.54	0.79	0.93	Front	0.17	0.68	Front
DNP-DL-Methionine	0.26	0.54	0.54	0.77	0.95	Front	0.36	0.95	Front
DNP-L-Phenylalanine	0.26	0.54	0.59	0.77	0.95	Front	0.39	0.97	Front
DNP-L-Proline	0.28	0.55	0.54	0.74	0.96	Front	0.26	0.77	Front
Group IV									
DNP-L-Valine	0.41	0.70	0.67	0.95	0.98	Front	0.39	0.96	Front
DNP-L-Isoleucine	0.50	0.75	0.74	0.97	0.98	Front	0.49	0.98	Front
DNP-L-Leucine	0.49	0.73	0.71	0.95	0.97	Front	0.56	0.97	Front

* Isooctane–chloroform–acetic acid. A = 100:50:5; B = 100:100:10; C = 100:150:5.
** Diethyl ether–acetic acid–water. A = 100:1:1; B = 100:3:3; C = 100:5:5.
*** Diethyl ether–methanol–7 *M* ammonium hydroxide. A = 100:6:2; B = 100:8:3; C = 100:10:4.

ether–methanol–7 *M* ammonium hydroxide (100:8:3). DNP-glutamine and asparagine are the most difficult to separate from each other. Compounds in Groups II, III and IV are separated by chromatography in the first dimension in isooctane–chloroform–acetic acid (100:100:10) and in the second dimension in ether–methanol–7 *M* ammonium hydroxide (100:6:2). DNP-phenylalanine and DNP-methionine are the most difficult compounds of Group IV to separate.

Discussion

Chromatography of DNP-derivatives on silica gel-impregnated glass paper overcomes many analytical problems. N-terminal amino acid identifications or the qualitative analysis of a mixture of DNP-amino acids in a submicrogram sample of an unknown can be carried out in less than one hour. Neither the reagents used in pre-

paring DNP-derivatives nor those used in hydrolyzing DNP-peptides hinder the resolving power of the glass paper technique. Upon completion of dinitrophenylation of a sample in bicarbonate or trimethylamine buffers, the reaction mixture may be spotted directly on the glass paper without preliminary extraction or purification of DNP-amino acids. Solutions of DNP-amino acids in 6 *N* HCl or in the various mixtures containing glacial acetic, formic and perchloric acids used in digesting DNP-peptides or DNP-proteins can be applied directly to the glass paper without seriously altering R_F values or quality of DNP-amino acid resolution. The three solvent systems presented here, an acidic, a basic and a non-aqueous acidic, offer wide selectivity and versatility in separating DNP-derivatives.

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1 F. SANGER, *Science*, 129 (1959) 1340.
2 H. FRAENKEL-CONRAT, J. I. HARRIS AND A. L. LEVY, *Methods of Biochemical Analysis*, Vol. II, Interscience, New York, 1955, p. 359.
3 J. G. HAMILTON, J. R. SWARTWOUT, O. N. MILLER AND J. E. MULDREY, *Biochem. Biophys. Res. Commun.*, 5 (1961) 226.

Received August 14th, 1964

J. Chromatog., 18 (1965) 192–194

The use of ninhydrin in the detection of cationic complexes of cobalt, nickel and chromium

In the course of the preparation[1–4] and purification of metal coordination compounds prior to the assessment of their biological properties[5], several methods of detection on paper and thin-layer chromatograms were tried. Of a number of reagents used, ninhydrin was found to give characteristic stains with the cationic complexes of cobalt, nickel and chromium.

All the complexes excepting compound No. 10 (Table I) were dissolved in water, applied to paper strips and developed by the descending method in a solvent system made up of *n*-butanol–acetic acid–water–pyridine (30:6:20:24, v/v). After staining with 0.2 % ninhydrin in acetone, the spots were visualised by heating the paper strips for 10–15 min at 80–90°. The water-labile compound No. 10 was suspended in acetone to which water was added drop by drop to get a fine suspension.

In spot tests carried out on strips of paper the cationic complexes listed in Table I reacted sensitively to ninhydrin, the range of detectability being 0.2–1.0 μg.