A MODIFIED TWO-DIMENSIONAL PAPER CHROMATOGRAPHIC SYSTEM FOR THE SEPARATION OF DNP-AMINO ACIDS

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(Received November 12th, 1959)

The 2,4-dinitrophenyl (DNP) derivatives of amino acids are used most often in studies on the determination of the structure of proteins and peptides. BISERTE, *et al.*[1] have recently reviewed the methods of paper chromatography for the separation of these amino acid derivatives. The two-dimensional paper chromatographic system employing a "toluene" solvent and 1.5 *M* phosphate buffer[2] is presently considered the most satisfactory for the separation of ether-soluble DNP-amino acids normally found in proteins. The "toluene" solvent, which is a mixture of toluene, pyridine, chloroethanol and ammonia, has been criticized on two points. It is not convenient to use this solvent in laboratories where either nitrogen or colorimetric ninhydrin determinations are being carried out routinely. Further, the two-phase "toluene" system requires several hours of equilibration before use and then the organic phase, used for the development of the chromatogram, is not stable. Other two-dimensional paper chromatographic systems which have been proposed have only substituted a different organic solvent for the "toluene" solvent[3,4].

In the solvent systems presently described, the phthalate buffer proposed by BLACKBURN AND LOWTHER[5] is used. The paper (Whatman No. 1, 22 in. × 18 in.) is dipped in 0.05 *M* phthalate buffer* at pH 6.0 and then air dried at room temperature. The solution of DNP-amino acids** is placed in one corner of the paper and the chromatogram equilibrated with an atmosphere of *n*-amyl alcohol and water for 2 hours. The chromatogram is then developed in a descending fashion for 15–18 hours with *n*-amyl alcohol saturated with 0.05 *M* phthalate buffer. The paper is allowed to air dry in the dark at room temperature. This usually requires about 3 to 6 hours. The chromatogram is then developed in the second direction for about 15 to 18 hours using 1.5 *M* sodium sulfate–0.05 *M* phthalate buffer at pH 6.0, in a descending direction. Usually it is necessary to allow the aqueous solvent to drip from the end of the paper chromatogram in order to have the amino acid derivatives move far enough for good resolution. The *n*-amyl alcohol–phthalate buffer solvent can be stored for at least six months, if kept in an air-tight container.

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* The 0.05 *M* phthalate buffer was prepared by mixing 50 ml of 0.1 *M* potassium acid phthalate and 45.45 ml of 0.1 *N* NaOH and diluting to 100 ml with CO_2-free water[5].

** All the DNP-amino acids used in this study were prepared by the method of SANGER[6] or that of LEVY AND CHUNG[7].

The movement of 19 DNP-amino acids and 2,4-dinitrophenylaniline in both solvent systems, in relation to the movement of 2,4-dinitrophenol, is given in Table I. The distances moved were measured from the point of origin to the center of the spot. Dinitrophenol moves about 20 cm from the origin after development for 17 hours

TABLE I

RELATIVE MOVEMENT OF DNP-AMINO ACIDS ON PAPER CHROMATOGRAMS

DNP-amino acid	*n-Amyl alcohol–phthalate buffer*	*Sulfate–phthalate buffer*
Aspartic acid	0.03	2.09
Glutamic acid	0.09	1.90
Cystine (di-DNP)	0.10	0.24
Serine	0.16	1.65
Histidine (di-DNP)	0.21	0.15
Glycine	0.22	0.98
Threonine	0.38	1.80
Alanine	0.58	1.26
Ornithine (di-DNP)	0.61	0.13
Proline	0.68	1.59
Lysine (di-DNP)	0.92	0.13
Methionine	0.98	1.03
Dinitrophenol	1.00	1.00
Arginine	1.02	1.02
Tyrosine (di-DNP)	1.10	0
Tryptophan	1.17	0.27
Valine	1.25	1.33
Phenylalanine	1.27	0.60
Isoleucine	1.43	1.35
Leucine	1.43	1.35
Dinitroaniline	1.66	0.42

with *n*-amyl alcohol–phthalate buffer solvent system at 23–25° (R_F = 0.5–0.55). In the 1.5 *M* sodium sulfate–phthalate buffer solvent system, dinitrophenol moves about 12 cm from the origin in 17 hours at 23–25° (R_F = 0.2–0.25).

The difference in the relative positions of the DNP-amino acids on the chromatograms between the presently described two-dimensional system and the systems previously reported is due mainly to the organic solvent used in the first dimension. The sulfate–phthalate buffer system gives a separation of the DNP-amino acids which is essentially the same as that obtained with the 1.5 *M* phosphate buffer. The sulfate–phthalate buffer solvent system was found to give more distinct spots with sharper boundaries for most of the DNP-amino acids than when the phosphate buffer solvent system was used, especially when the amino acid derivatives had first been separated with the use of the *n*-amyl alcohol–phthalate buffer system.

The general distribution of the DNP-amino acids on the chromatogram developed with *n*-amyl alcohol–phthalate buffer closely resembles the distribution obtained with butan-2-ol–0.05 *M* phthalate buffer at pH 6.0 [5]. The R_F of the DNP-amino acids which move more slowly than dinitrophenol is less when the *n*-amyl alcohol solvent is used than when the butan-2-ol solvent is used. Furthermore, the DNP-derivatives of

glycine, serine, threonine and alanine can be completely resolved on a unidimensional chromatogram with the *n*-amyl alcohol–phthalate buffer system.

The presently described solvent systems cannot be used to separate DNP-methionine, α-DNP-arginine and dinitrophenol. However, α-DNP-arginine is not found in the DNP-amino acids which are extractable from aqueous media with ether; dinitrophenol can be removed by sublimation or by bleaching with HCl fumes.

SUMMARY

The two-dimensional separation of DNP-amino acids by solvent systems which appear to overcome most of the shortcomings of previously described solvent systems is described. The organic solvent consists of *n*-amyl alcohol saturated with 0.05 *M* phthalate buffer at pH 6.0; the aqueous solvent consists of 1.5 *M* sodium sulfate containing 0.05 *M* phthalate buffer (pH = 6.0).

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