Journal of Chromatography, 154 (1978) 247–250

CHROM. 10,884

Note

Solvent systems for thin-layer chromatography of Dns-amino acids on polyamide sheets

ROBERT M. METRIONE

Department of Biochemistry, Jefferson Medical College, Thomas Jefferson University, 1020 Locust Street, Philadelphia, Pa. 19107 (U.S.A.)

(First received November 17th, 1977; revised manuscript received January 16th, 1978)

The chromatographic procedure which is most often employed in conjunction with the Edman-Dns technique[1] for sequencing peptides is two-dimensional thin-layer chromatography on polyamide sheets using the water–formic acid (200:3)–benzene–acetic acid (9:1) solvent system of Woods and Wang[2]. These workers also listed three other solvent systems which they termed "effective" but these have not found widespread use. In addition, Hartley[3] has described a solvent system (1 *N* ammonia–ethanol, 1:1) which is capable of separating basic Dns-amino acids when used as a "third solvent" on the two-dimensional chromatograms. A solvent has been described[4], also used as a third solvent, to be used for the separation of Dns derivatives of acidic amino acids and hydroxyl amino acids. Of these solvents, figures have been presented for the two-dimensional system[2,5] but no figures or tables of R_F values have appeared for all Dns-amino acids in any of the other systems.

It should be pointed out that far more solvent systems have been developed for the separation of Dns-amino acids on silica gel than on polyamide sheets[6–13]. Some of these systems are very effective and solvents can be selected for general separations of Dns derivatives or for the separation of any group of Dns-amino acids. However because the Dns-amino acids are rapidly destroyed on silica gel but are much more stable on polyamide[5,14], and because very low amounts of Dns-amino acids can be detected on polyamide sheets cut as small as 5 cm square, polyamide sheets have now largely supplanted silica gel for the identification of Dns-amino acids. Our discussion will, therefore, be limited to those solvents which are known to be useful on this support.

We considered it useful to determine the R_F values of the Dns derivatives of commonly occurring amino acids in all the reported systems and to develop new solvent systems which would yield useful separations of basic acidic and hydroxyl derivatives in the presence of other amino acids without resorting to the "third solvent" systems of Hartley[3] and Magnusson[4].

MATERIALS

The polyamide sheets were microlayer preparations obtained from Brinkmann Instruments (Westbury, N.Y., U.S.A.). These were trimmed to 9 × 9 cm before use.

The Dns amino acids were obtained from Schwarz/Mann (Orangeburg, N.Y., U.S.A.) or were synthesized according to Gray[5]. The solvents were analytic grade reagents.

RESULTS AND DISCUSSION

Table I lists the R_F values for eighteen commonly occurring Dns-amino acids in several solvent systems. The relative R_F values in the benzene–acetic acid system (A) of Woods and Wang[1] and the new toluene-containing systems (B and C) are very similar. The advantage of the new systems is the substitution of toluene for benzene resulting in a much less toxic solvent. Since these solvents are used routinely in many laboratories a long-term low-level inhalation by workers can reasonably be expected. Such exposure to benzene has been shown to be capable of causing bone-marrow depression, aplastic anemia and leukemia[15]. In some applications, where prolonged exposure to the solvents is likely, it may be desirable to substitute one of the toluene systems for the benzene system*.

TABLE I

R_F VALUES FOR Dns-AMINO ACIDS IN VARIOUS SOLVENT SYSTEMS ON POLYAMIDE SHEETS

Solvent systems: A = benzene–acetic acid (9:1); B = toluene–acetic acid (9:1); C = toluene–ethanol–acetic acid (17:1:2); D = water–formic acid (200:3); E = water–ethanol–ammonium hydroxide (17:2:1); F = ethyl acetate–ethanol–ammonium hydroxide (20:5:1); G = water–ethanol–ammonium hydroxide (14:15:1); H = *n*-heptane–*n*-butanol–acetic acid (3:3:1); I = chlorobenzene–acetic acid (9:1); J = ethyl acetate–methanol–acetic acid (20:1:1). All of the proportions are based on volume.

Dns-amino acid	*R_F in solvent system*									
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
1 Ala	0.53	0.48	0.49	0.69	0.69	0.57	0.81	0.68	0.43	0.79
2 Arg	0.05	0.03	0.03	0.91	0.39	0.09	0.76	0.22	0.01	0.06
3 Asp	0.08	0.07	0.10	0.69	0.88	0.10	0.88	0.37	0.12	0.19
4 Cys	0.03	0.03	0.04	0.19	0.43	0.22	0.78	0.09	0.03	0.06
5 Glu	0.15	0.10	0.15	0.66	0.88	0.02	0.88	0.34	0.05	0.30
6 Gly	0.32	0.21	0.32	0.69	0.63	0.48	0.80	0.48	0.28	0.69
7 His	0.07	0.05	0.13	0.96	0.76	0.32	0.84	0.36	0.06	0.18
8 Ile	0.77	0.54	0.65	0.40	0.57	0.71	0.78	0.76	0.60	0.84
9 Leu	0.70	0.49	0.59	0.34	0.57	0.71	0.78	0.75	0.54	0.80
10 Lys (mono)	0.35	0.21	0.38	0.22	0.09	0.63	0.72	0.58	0.09	0.79
11 Lys (di)	0.53	0.37	0.48	0.78	0.69	0.35	0.82	0.40	0.39	0.76
12 Met	0.52	0.36	0.51	0.43	0.59	0.68	0.80	0.62	0.55	0.81
13 Phe	0.57	0.38	0.53	0.31	0.43	0.68	0.77	0.62	0.51	0.81
14 Pro	0.85	0.66	0.71	0.55	0.74	0.46	0.84	0.75	0.69	0.90
15 Ser	0.12	0.07	0.16	0.81	0.71	0.49	0.82	0.42	0.10	0.44
16 Thr	0.15	0.10	0.26	0.81	0.74	0.57	0.82	0.56	0.16	0.56
17 Tyr	0.63	0.47	0.61	0.00	0.00	0.84	0.73	0.65	0.58	0.91
18 Val	0.72	0.56	0.61	0.47	0.67	0.71	0.81	0.80	0.61	0.88
19 Dns–OH	0.00	0.01	0.00	0.51	0.54	0.16	0.74	0.00	0.04	0.04
20 Dns–NH_2	0.51	0.38	0.47	0.71	0.17	0.96	0.49	0.60	0.40	0.91

* *Editor's note*: Please note that toluene is also toxic.

The other new solvents presented in Table I offer the advantage of versatility. The basic solvents E and F give a good separation of basic Dns-amino acids. While the separation of aliphatic and aromatic derivatives is not very good it is much better than in solvent G the ethanol–1 *N* ammonia system of Hartley[3]. These are the first basic solvents of general utility to be described for the separation of Dns-amino acids on polyamide sheets.

In situations where it is desirable to obtain a two-dimensional chromatogram of a sample it is now possible to run one dimension in an acidic solvent and the other in a basic solvent. Such chromatograms are shown in Fig. 1B, C, D and F. Using these systems the separation of basic amino acid derivatives, in the presence

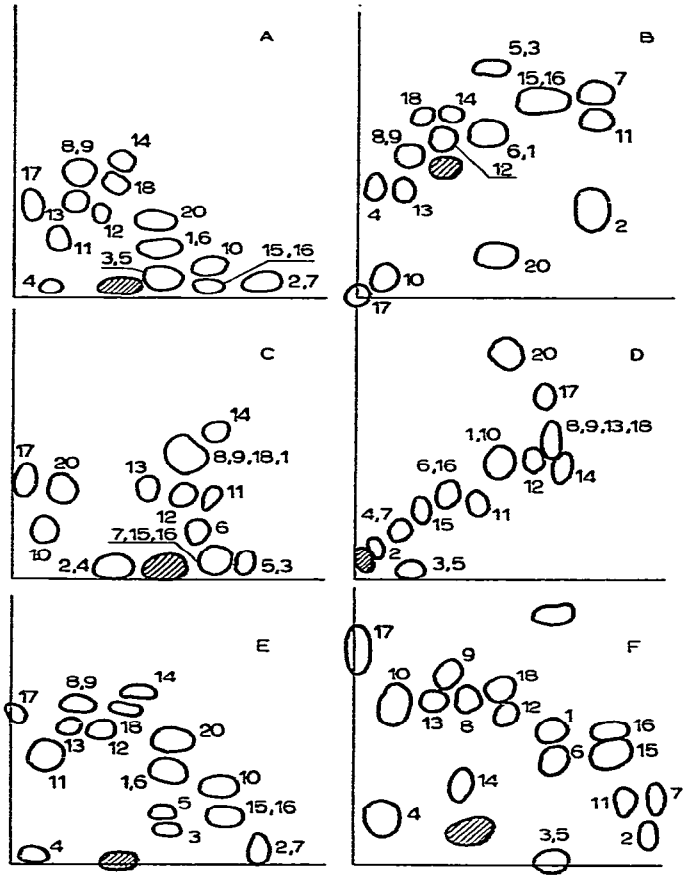

Fig. 1. Thin-layer chromatography of Dns-amino acids on polyamide sheets. Solvents: A, horizontal, water–90% formic acid (200:3), vertical, toluene–acetic acid (9:1); B, horizontal, water–90% formic acid (200:3), vertical, water–ethanol–ammonium hydroxide (17:2:1); C, horizontal, water–ethanol–ammonium hydroxide (17:2:1), vertical, toluene–acetic acid (9:1); D, horizontal, toluene–ethanol–acetic acid (17:1:2), vertical, ethyl acetate–ethanol–ammonium hydroxide (20:5:1); E, horizontal, water–90% formic acid (200:3), vertical toluene–ethanol–acetic acid (17:1:2); F, horizontal, water–90% formic acid (200:3), vertical, ethyl acetate–ethanol–ammonium hydroxide (20:5:1). All of the proportions are on a volume basis. The numbers in the figure represent the Dns-amino acids listed in Table I. Approximately 0.5–1.0 nmole of each derivative was applied.

of other amino acids, does not require the use of a third solvent. Only system E gives a good separation of the acidic Dns-amino acids without the use of a third solvent, although a slight separation is also seen with system D. Dns-Threonine and Dns-serine are also resolved in this system. System D would appear to be of general utility comparable to the standard system of Woods and Wang[1] or system A. Thus a solvent combination can be chosen on the basis of the amino acid content of the peptide being studied.

REFERENCES

1 W. R. Gray and B. S. Hartley, *Biochem J.*, 89 (1963) 379.
2 K. R. Woods and K. T. Wang, *Biochem. Biophys. Acta*, 133 (1967) 369.
3 B. S. Hartley, *Biochem. J.*, 119 (1970) 805.
4 S. Magnusson, quoted in C. P. Milstein and A. Feinstein, *Biochem. J.*, 107 (1968) 559.
5 W. R. Gray, *Methods Enzymol.*, 25 (1972) 137.
6 N. Seiler and J. Weichman, *Experientia*, 20 (1964) 559.
7 D. Morse and B. L. Horecker, *Anal. Biochem.*, 14 (1966) 429.
8 K. Crowshaw, S. Jessup and D. W. Ramwell, *Biochem. J.*, 103 (1967) 79.
9 J. P. Zanetta, G. Vincendon, P. Mandel and G. Combos, *J. Chromatogr.*, 51 (1970) 441.
10 N. Seiler, *Methods Biochem. Anal.*, 18 (1970) 259.
11 J. Rosmus and Z. Deyl, *Chromatogr. Rev.*, 13 (1970) 163.
12 V. A. Spivak, M. I. Levjant, S. P. Katrukha and J. M. Varshavsky, *Anal. Biochem.*, 44 (1971) 503.
13 R. S. Fager and C. B. Kutina, *J. Chromatogr.*, 76 (1973) 268.
14 M. I. Pouchan and E. J. Passeron, *Anal. Biochem.*, 63 (1975) 585.
15 R. Snyder and J. J. Kocsis, *CRC Critical Rev. Toxicol.*, 3 (1975) 265.