Journal of Chromatography, 115 (1975) 665–669

CHROM. 8621

Note

Centrifugal chromatography of free and dansylated amino acids

MARGARET J. WYSZYNSKI and NORMAN E. HOFFMAN
Department of Chemistry, Marquette University, Milwaukee, Wisc. 53233 (U.S.A.)
(First received May 22nd, 1975; revised manuscript received July 21st, 1975)

Centrifugal chromatography on short columns of microparticulate silica gel has been applied successfully to several types of compounds[1–8]. The greatest emphasis has been on lipid separation. We decided to investigate the separation of amino acids in a few solvent systems to see if these very polar compounds could be separated by this technique with some advantage over the more conventional thin-layer chromatography (TLC). Dansylated amino acids were also studied.

The column is completely wetted by solvent at the beginning of a separation by centrifugal chromatography, and, therefore, R_F values cannot be measured. We chose to refer movement to a fast moving dye or the fastest moving amino acid, and defined the parameter R_X:

$$R_X = \frac{\text{distance travelled by the amino acid}}{\text{distance travelled by the internal standard}}$$

The study we wish to report emphasizes separations by centrifugal chromatography as measured by R_X values.

EXPERIMENTAL

Equipment and materials

An Ivan Sorvall (Norwalk, Conn., U.S.A.) GLC-1 centrifuge with a HL-4 rotor and an Ivan Sorvall Centri-Chrom apparatus were used. Silica was obtained from Ivan Sorvall or Philadelphia Quartz (Philadelphia, Pa., U.S.A.) (QUSO G-32). It was dried for 12 h at 120° and then placed in a dry box. Free amino acids and dansylated amino acids were purchased from Pierce (Rockford, Ill., U.S.A.). Ninhydrin, 2-butanone, 1-butanol, chloroform, and *tert.*-amyl alcohol were obtained from Aldrich (Milwaukee, Wisc., U.S.A.); pyridine, methanol, 2-propanol, ammonia, and acetic acid from Fisher Scientific (Pittsburgh, Pa., U.S.A.).

Solvent compositions were: (1), 2-butanone–pyridine–glacial acetic acid–water (70:15:15:10); (2), chloroform–methanol–ammonia (17%) (8:8:1); (3), 1-butanol–2-butanone–formic acid–water (60:105:15:20); (4), chloroform–1-butanol–acetic acid (60:40:5); (5), chloroform–*tert.*-amyl alcohol–acetic acid (160:60:1).

Procedure

Glass columns, which were silanized daily with dimethyldichlorosilane in

toluene (2:100, v/v), were packed with a slurry of silica in the development solvent to be used for separation. Columns were 3 mm I.D., and packing was done by centrifugation at 3000 rpm or 1500 *g*. Packing times were: for solvents 1, 2, 4 and 5, 15 min; for solvent 3, 20 min.

After packing, the top of the column was reamed and the loading disc on which the sample to be separated had been placed was added to the top. Amino acids (1 mg) were dissolved in 1 ml of 2-propanol containing 2 or 3 drops of 12 *M* hydrochloric acid. The sample consisted of 0.1–4 μl of this solution depending on the experiment and the amino acid. In some runs the sample was placed directly on the column or on the porous stopper.

All separations were developed at 3000 rpm or 1500 *g*. Development times were: solvents 1 and 3, 20 min; 2 and 5, 15 min; 4, 13 min. After development the columns were extruded with the Sorvall extrusion apparatus. The silica columns were then heated in a 120° oven for at least 10 min. After drying, columns with free amino acids were sprayed with a solution of 400 mg ninhydrin and 1.5 ml collidine in 100 ml 95% ethanol. The columns were dried at 120° for 10 min after spraying. Dansylated amino acids were visualized after drying with a ultraviolet lamp (365 nm) directly without spraying.

Internal standard dyes were applied as the amino acids were but separately in concentration of 1 mg/ml.

TABLE I

R_x VALUES FOR AMINO ACIDS IN THREE SOLVENTS

For solvent 1, R_x is relative to Sudan black; for solvent 2, R_x is relative to phenylalanine; for solvent 3, R_x is relative to fluorescein.

Amino acid	$R_x \pm$ *standard deviation*		
	Solvent 1	*Solvent 2*	*Solvent 3**
Alanine	0.49 ± 0.01	0.79 ± 0.02	0.75
Arginine	0.16 ± 0.01	0.07 ± 0.01	0.02
Asparagine	0.43 ± 0.01	0.58 ± 0.02	0.37
Aspartic acid	0.28 ± 0.01		0.63
Glutamic acid	0.44 ± 0.02		0.88
Glutamine	0.44 ± 0.01	0.66 ± 0.01	0.46
Glycine	0.39 ± 0.01	0.64 ± 0.02	0.51
Histidine	0.22 ± 0.01	0.76 ± 0.02	0.02
Hydroxyproline	0.43 ± 0.01	0.57 ± 0.02	0.59
Isoleucine	0.71 ± 0.01	0.93 ± 0.01	0.89
Leucine	0.71 ± 0.01	0.95 ± 0.01	0.89
Lysine	—	0.08 ± 0.02	0.02
Methionine	0.67 ± 0.01	0.96 ± 0.01	0.86
Phenylalanine	0.72 ± 0.01	1.00	0.88
Proline	0.43 ± 0.01	0.54 ± 0.02	0.62
Serine	0.45 ± 0.01	0.61 ± 0.02	0.48
Threonine	0.54 ± 0.01	0.79 ± 0.02	0.64
Tryptophan	0.72 ± 0.01	0.90 ± 0.01	0.91
Tyrosine	0.69 ± 0.01	0.92 ± 0.01	0.88
Valine	0.64 ± 0.01	0.94 ± 0.01	0.88

* Less than 5 determinations of R_x were made; therefore, no standard deviation was calculated.

RESULTS AND DISCUSSION

Table I shows R_X values for several amino acids. Reproducibility of these values is very good. In order to visually resolve two bands a ΔR_X of about 0.1 is necessary. Thus many amino acids cannot be resolved by one solvent alone.

The work of Fahmy *et al.*[9] served as a guide in developing solvents 1 and 2. Modifications in the component ratios of Fahmy *et al.* had to be made to avoid serious band spreading (greater than 3 mm). Solvent 3 was developed by us with the intention of having a strong acid, formic acid, present. In general, solvents with high concentrations of hydroxylated components were avoided because these produced soft columns that slowly collapsed during centrifugation.

When solvent ratios were varied, relative migrations sometimes changed from those reported by Fahmy *et al.* This was the result of the solvent change and not some peculiarity of the microparticulate silica stationary phase. When the amino acids concerned were separated on a thin-layer plate by the altered solvent, the same relative migration change occurred.

In searching for dyes we sought two properties. The dyes chosen had bandwidths less than 4 mm and they migrated faster than any amino acid chromatographed.

Table II gives the microgram amount of amino acid that is just detectable when the ninhydrin reagent described above is used. The detectability is a function of the solvent, and the minimum amounts necessary for detection is highest, in general, with solvent 2.

Table III shows R_X values of dansylated amino acids. Solvent 4 is the same as

TABLE II

DETECTABILITY OF AMINO ACIDS IN THREE SOLVENTS

Amino acid	*Detectability (μg)*		
	Solvent 1	*Solvent 2*	*Solvent 3*
Alanine	0.3	0.3	0.2
Arginine	1.0	0.8	0.4
Asparagine	1.0	3.0	0.8
Aspartic Acid	0.5	—	0.1
Glutamic Acid	1.0	—	0.3
Glutamine	0.7	2.5	0.3
Glycine	0.2	0.5	0.2
Histidine	0.5	2.7	0.4
Hydroxyproline	0.5	2.2	0.3
Isoleucine	0.4	0.3	0.3
Leucine	0.4	0.3	0.2
Lysine	—	0.4	0.2
Methionine	0.3	0.7	0.3
Phenylalanine	0.8	0.3	0.4
Proline	0.3	0.3	0.2
Serine	0.2	2.5	0.2
Threonine	0.3	3.3	0.5
Tryptophan	0.3	0.5	0.3
Tyrosine	0.2	0.3	0.2
Valine	0.3	0.3	0.2

TABLE III

R_X VALUES FOR DANSYL AMINO ACIDS IN TWO SOLVENTS

For solvent 1, R_X is relative to tetrabromophenolphthalein ethyl ester; for solvent 2, R_X is relative to dansyl isoleucine.

Dansyl amino acid	R_X	
	Solvent 4	*Solvent 5*
Alanine	0.89	0.45
Arginine	0.00	0.00
Asparagine	0.14	0.00
Aspartic acid	0.23	0.00
Cysteine	0.14	0.00
Cystine	0.03	0.00
Glutamic acid	0.56	0.00
Glycine	0.78	0.14
Histidine	0.39	0.00
Hydroxyproline	0.61	0.06
Isoleucine	0.95	1.00
Leucine	0.93	0.69
α-Lysine	0.00	0.00
DLysine	0.91	0.38
ε-Lysine	0.00	0.00
Methionine	0.91	0.43
Norleucine	0.94	0.69
Norvaline	0.93	0.72
Phenylalanine	0.92	0.53
Proline	0.91	0.55
Serine	0.48	0.00
Threonine	0.56	0.00
Tryptophan	0.88	0.33
Dityrosine	0.91	0.46
O-Tyrosine	0.00	0.00
Valine	0.93	0.93

that used by Stehelin and Duranton[10]. Varying the component ratios had no beneficial effect on resolution. Solvent 5 contained a little more chloroform than that used by Morse and Horecker[11]. The increase in chloroform content improved resolution.

Table III shows that solvent 4 separates the more polar amino acids but not the non-polar ones, whereas solvent 5 separates non-polar amino acids but not polar ones. We made one practical observation: it is necessary to apply the sample to the porous stopper because the dansyl derivatives were strongly adsorbed by the loading disc.

In conclusion the use of centrifugal chromatography for separation of amino acids and their dansylated derivatives has a time advantage over conventional TLC. If plate preparation time is included, then centrifugal chromatographic separations take much less time. There seems to be no special resolution advantage of centrifugal chromatography over TLC. There is a disadvantage in centrifugal chromatography compared to TLC when limits of detectability are considered.

REFERENCES

1 H. J. McDonald, L. V. McKendell and E. W. Bermes, Jr., *J. Chromatogr.*, 1 (1958) 259.
2 Z. Deyl, J. Rosmus and M. Pavlíček, *Chrom. Rev.*, 6 (1964) 19.
3 E. Piel, *Anal. Chem.*, 38 (1966) 670.
4 E. Ribi, R. L. Anacker, W. R. Barclay, W. Brehmer, G. Middlebrook, K. C. Milner and D. F. Tarmina, *Ann. N.Y. Acad. Sci.*, 154 (1968) 41.
5 E. Ribi, C. J. Filz, K. Ribi, G. Goode, W. Brown, M. Niwa and R. Smith, *J. Bacteriol.*, 102 (1970) 250.
6 E. Ribi, C. J. Filz, G. Goode, S. M. Strain, K. Yamamoto, S. C. Harris and J. H. Simmons, *J. Chromatogr. Sci.*, 8 (1970) 577.
7 C. Wynne-Roberts and D. Davis, *J. Chromatogr. Sci.*, 11 (1973) 406.
8 R. L. Anacker, J. H. Simmons and E. Ribi, *J. Chromatogr.*, 62 (1971) 93.
9 A. R. Fahmy, A. Niederwieser, G. Pataki and M. Brenner, *Helv. Chim. Acta*, 44 (1961) 2022.
10 D. Stehelin and H. Duranton, *J. Chromatogr.*, 43 (1969) 93.
11 D. Morse and B. Horecker, *Anal. Biochem.*, 14 (1966) 429.