CHROM. 4112

## The extension of thin-layer electrophoresis on cellulose to the identification of DNS-amino acids

The ease of preparation and greater sensitivity of the DNS* amino acids compared to DNP derivatives favor their utilization in many protein sequence studies. Although the expense involved in determining these residues employing high-voltage electrophoresis[1] has been reduced using chromatography on paper[2] or silica gel[3–5], several systems are necessary for accurate identification. While similar mobilities have been obtained in many comparative studies (*e.g.*, ref. 6) of thin-layer cellulose and paper chromatography, the lack of agreement encountered with different types of thin-layer cellulose and filter paper in electrophoresis led us to further investigate this phenomenon. We have found that the electrophoretic mobility on Eastman thin-layer cellulose sheets parallels that found using filter paper and that these values are quite different from those obtained using various other thin-layer adsorbents.

*Materials and methods*

Eastman Kodak Cellulose Chromagram Sheets (20 × 20 cm) without fluorescent binder were used throughout this investigation except as noted. A Canalco Model 1400 power supply was employed in conjunction with a water-cooled electrophoresis apparatus modeled after KATZ AND LEWIS[7]. Standard solutions of DNS amino acids were purchased from Mann Research Laboratories of prepared according to GRAY[8].

The adsorbent was sprayed with a pH 4.4 pyridine–acetic acid buffer (0.4 % pyridine and 0.8 % acetic acid) at a distance of 12–14 in. To insure proper moisture 6.5–7 ml of buffer are used per 20 × 20 cm of sheet. Samples (0.5–10 mmoles in 1–5 $\mu$l of acetone or 50 % aqueous pyridine) were then spotted at their respective origins as noted in Fig. 1. The sheet was placed on the apparatus and Whatmann No. 3 MM

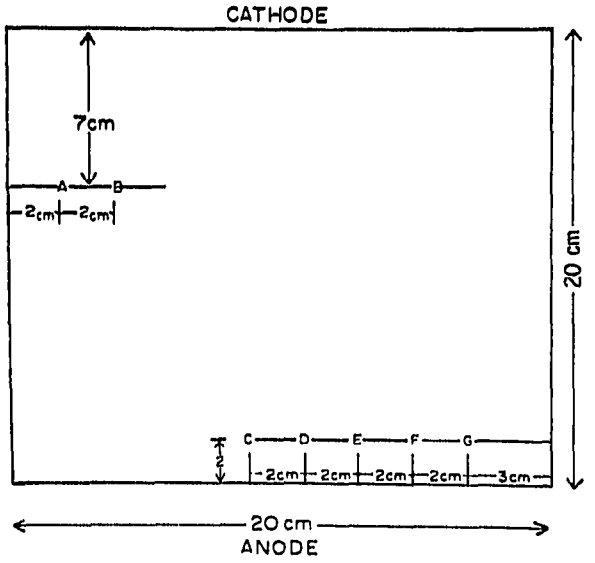


Fig. 1. Arrangement of thin-layer sheet for sample application. Letters A through G denote sample application. The contents of each are as noted under Fig. 2.

* DNS = diaminonaphthaline sulfonyl; DNP = dinitrophenyl.

filter paper previously wetted with buffer and blotted was placed at each end as wicks and 1000 V was applied for 1 h. If required, an additional 1-h electrophoresis was employed. To avoid extensive evaporation a sheet of glass was placed over the adsorbent plate which was separated slightly by supports of lucite or glass. The temperature was maintained at 12–15° using either an ice bath or a regulated refrigeration unit.

Following electrophoresis or chromatography the cellulose sheet was dried at 60–70° for 10-15 min or until the solvent was completely evaporated and exposed to ammonia fumes in a desiccator for 5–6 min.

Visualization of the derivatives was made possible by their fluorescence under U.V. light. Under the conditions employed they appeared yellow while the major by-product, diaminonaphthalenesulfonic acid, fluoresced blue.

*Results and discussion*

As indicated in Fig. 2 electrophoresis at pH 4.4 at 12–15° for 1 h at 1000 V allows one to clearly distinguish many of the common amino acids. Under these conditions the derivatives of dansyl histidine, arginine, glutamic acid, aspartic acid, tryptophan, methionine sulfoxide, cysteine, mono- and di-lysine and tyrosine are easily recognizable. To better identify those amino acids migrating between glycine and α-DNS-tyrosine an additional period of 1 h was necessary (Fig. 3). Higher voltages with concomitantly reduced running time were possible but not employed as this necessitated more elaborate equipment.

Although the temperature employed was varied from −10° to 28° a working range of 12–15° afforded maximum resolution. Below approximately 5° an increased ratio of migration was noted when comparing the neutral and acidic amino acids to

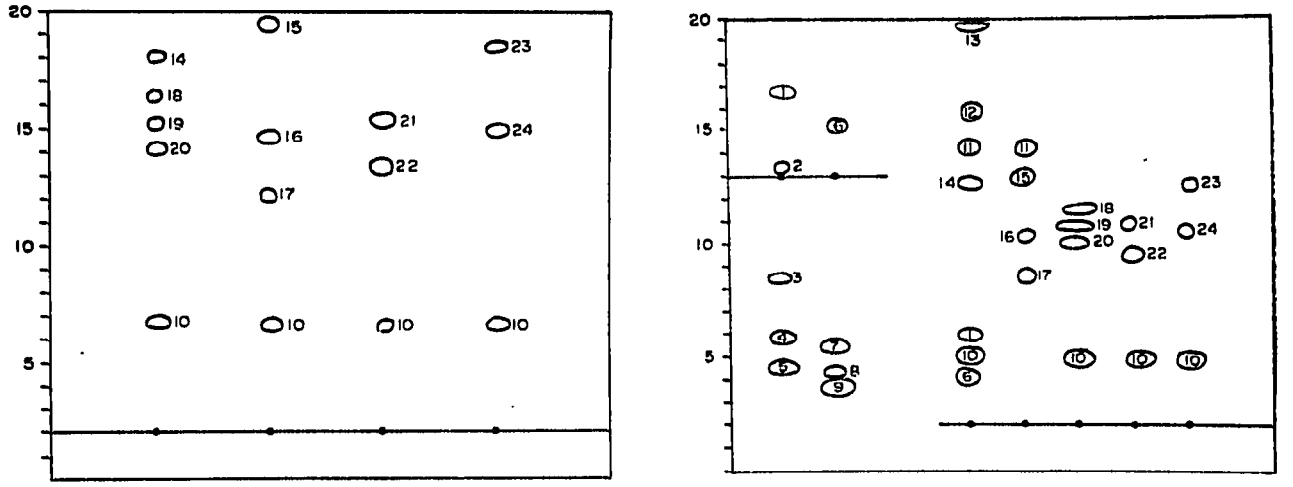


Fig. 2. Diagrammatic separation of dansyl amino acids after 1 h of migration. The amino acids are numbered as follows: 1 = α-tyrosine, 2 = dityrosine, 3 = *o*-tyrosine, 4 = histidine, 5 = arginine, 6 = dilysine, 7 = ε-lysine, 8 = DNS-$NH_2$, 9 = α-lysine, 10 = tryptophan, 11 = glutamic acid, 12 = aspartic acid, 13 = cysteine, 14 = DNS-OH, 15 = glycine, 16 = threonine, 17 = phenylalanine, 18 = alanine, 19 = leucine, 20 = valine, 21 = proline, 22 = isoleucine, 23 = serine, and 24 = hydroxyproline.

Fig. 3. Diagrammatic separation of the neutral amino acids after 2 h of migration. The numbering follows Fig. 2.

the basic. Above 17–18° heating effects including excessive evaporation and considerable streaking tended to decrease the resolution and reproducibility.

Unlike the direct relationship which was found between migration on Eastman Chromagram sheets and that on Whatmann filter papers numerous differences have been disclosed using manually prepared Cellulose 300 MN (Brinkman) (Arnott & Ward).

Most important was the extreme anodal migration of the basic and to a lesser extent most of the neutral dansyl amino acids. Brinkman's prepared adsorbent gave results identical to manually prepared cellulose layers and therefore at great variance with filter paper and Eastman thin layers. In addition this sheet had a lower moisture capacity (5.5–6 ml for optimal resolution) and a greater current fluctuation than the Eastman sheet.

The occasional ambiguities encountered in thin-layer electrophoresis on cellulose of dansyl amino acids were found to be of lower order than those observed with standard filter paper electrophoresis. In those instances where the need for further resolution was required the area containing the unresolved band was moistened and transferred to another sheet with gentle pressure for further electrophoretic separation.

Electrophoresis was also attempted on Eastman Silica Gel Chromagram Sheets but this was discontinued as it not only required prior washing to neutralize the acidity but also longer times to produce separations comparable to those obtained with the cellulose layers. In those instances where chromatography was performed the Eastman product was found to give slightly higher $R_F$ values, regardless of the solvents employed, than either filter paper or manually prepared adsorbent.

The author thanks Dr. P. STELOS for his aid in this investigation, Miss JOANNA DANEMAN for her technical competence, and Miss ROSANNA VELASQUEZ for her secretarial assistance.

This work was supported by USPHS grant AI 07137 and a USPHS fellowship from the National Institutes of General Medical Sciences.

*Department of Microbiology, Hahnemann Medical College, Philadelphia, Pa. 19102 (U.S.A.)* BRUCE P. SLOAN

1 W. R. GRAY AND B. S. HARTLEY, *Biochem. J.*, 89 (1963) 60P.
2 A. A. BOULTON AND I. E. BUSH, *Biochem. J.*, 92 (1964) 11P.
3 D. MORSE AND B. L. HORECKER, *Anal. Biochem.*, 14 (1966) 429.
4 Z. DEYL AND J. ROSMUS, *J. Chromatog.*, 20 (1965) 514.
5 N. SEILER AND J. WEICHMANN, *Experientia*, 20 (1964) 559.
6 J. W. BURNS AND N. A. TURNER, *J. Chromatog.*, 30 (1967) 469.
7 S. KATZ AND A. LEWIS, *Anal Biochem.*, 17 (1966) 300; 16 (1964) 327.
8 W. R. GRAY, in C. H. W. HIRS (Editor), *Methods in Enzymology*, Vol. XI, Academic Press, New York, 1967.
9 M. S. ARNOTT AND D. N. WARD, *Anal. Biochem.*, 21 (1967) 50.

First received February 25th, 1969; revised manuscript received April 2nd, 1969