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Note

Paper electrophoresis of the dansyl derivatives of amino acids and amines

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Paper electrophoresis has been widely used for separating free amines[1–3] and free amino acids[4–6], but electrophoresis of their derivatives has been limited to the study of dinitrophenyl[7] and dansyl amino acids[8,9]. Gray and Hartley[8,9] separated the dansyl derivatives of all the protein amino acids except for those of serine, proline and alanine by high-voltage electrophoresis at pH 4.4 (Whatman 3 mm paper, 80 V cm^{-1} 3 h) and completely resolved the remainder at pH 12.7 (30 V cm^{-1} 2 h). Klimek *et al.*[10] determined 18 dansyl protein amino acids at pH 12.7 using the spot area approach. There are no reports of the electrophoresis of dansylated amines (defined as amino and imino compounds lacking COOH groups) or of dansylated non-protein amino acids.

The present work had its origin in the isolation and characterisation of minute quantities of unusual tissue amines as their dansyl derivatives[11]. These derivatives are much easier to purify than the original amines because they chromatograph more cleanly, can be separated from salts by solvent extraction and can be detected at much lower levels. It was hoped that electrophoresis might provide an alternative method for purifying these derivatives and would help to identify the functional groups of the isolates obtained. In particular, by using buffers having a wide range of pH values it was hoped to distinguish between the derivatives of amines and those of basic amino acids since both classes of parent compound appear in the same basic nitrogen fraction[12].

EXPERIMENTAL

Electrophoresis equipment

Low-voltage electrophoresis: a Shandon Southern Kohn Model U77 electrophoresis tank was used with a Shandon 500 V/50 mA power pack.

High-voltage electrophoresis: a Shandon high-voltage electrophoresis chamber Model Q11 was operated with a Shandon 5 kV/200 mA power supply.

Preparation of buffers

pH 2.0: 90% (v/v) formic acid, 16 ml; glacial acetic acid, 74 ml; water, 910 ml. pH 4.0: 90 ml 0.2 *M* sodium acetate + 410 ml 0.2 *M* acetic acid. pH 5.7: 250 ml 0.1 *M* sodium dihydrogen phosphate + 18.0 ml 0.1 *M* sodium hydroxide + 232 ml

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water. pH 9.4: 50 ml 0.1 *M* sodium carbonate + 450 ml 0.1 *M* sodium bicarbonate. pH 10.2: 450 ml 0.1 *M* sodium carbonate + 50 ml 0.1 *M* sodium bicarbonate.

Preparation of dansyl derivatives

This was a modification of the method developed by Seiler and Wiechmann[13]. To 200 μl aqueous amine or amino acid (2 mg ml^{-1}) was added 400 μl 5-dimethylaminonaphthalene-1-sulphonyl chloride (dansyl chloride, 5 mg ml^{-1} in acetone). After leaving for about 15 h in darkness at 20 ± 5°C the amino acid reaction products were electrophorised without further treatment. However, mixtures containing dansylated amines were further reacted with 400 μl aqueous proline (15%) for 30 min at 20°C, followed by extraction with 2 × 2.5 ml ethyl acetate. These organic fractions were combined and evaporated to dryness in a stream of air at 50°C, the residue being redissolved in 200 μl ethyl acetate for spotting.

Paper electrophoresis

Dansylated samples (2 μl = 4 μg free amino/imino compound) were spotted on Whatman No. 1 paper together with aqueous samples of glucose (20 μg) to monitor mass buffer flow, and potassium chloride (20 μg) to act as a standard so relative mobilities at different pH values could be calculated. K^+ was selected as it remained soluble and completely ionised in all buffers.

The dansyl amino acids were routinely electrophorised for 30 min at 300 V and the dansyl amines for 2 h at 300 V (paper size 10–20 cm wide × 18 cm between wicks). In the latter case dansyl methylamine was used as a secondary standard and its mobility was related to that of K^+ in a separate experiment, because conditions giving optimum separation of dansyl amines ran K^+ off the paper.

After electrophoresis the papers were dried and the dansyl derivatives detected by their fluorescence under 366 nm UV light.

Glucose[14] was detected by spraying the relevant parts of the electrophoretograms with 1% aqueous potassium permanganate containing 2% sodium carbonate. Glucose appeared as a brown spot on a purple background, changing to grey on a brown background.

Potassium[15] was detected by spraying the relevant parts of the electrophoretogram with a reagent prepared in the following way: 5.7 g hydrated cobalt acetate, 8.1 g hydrated lead(II) acetate, 10 g sodium nitrite and 2 ml glacial acetic acid were dissolved in 75 ml water and mixed with 20 ml methanol. K^+ appeared as a yellow spot on a brown background. Electrophoretograms run in the pH 9.4 and 10.2 buffers were dipped in glacial acetic acid and dried before treating with this cobalt hexanitrite reagent.

RESULTS AND DISCUSSION

The results are given in Tables I–III and illustrated by Fig. 1. The standardized data shows actual mobilities, adjusted to average conditions as judged by the movement of the standards. This first set of figures has been corrected by subtracting mass buffer flow and then calculating the distance each dansyl derivative would have moved as K^+ electrophoresed 100 mm.

Dansyl derivatives are normally handled in organic solvents like benzene, ethyl acetate and acetone so it is perhaps surprising that all except seven proved sufficiently

TABLE I

ELECTROPHORETIC MOBILITIES OF DANSYL AMINO ACIDS

Negative values indicate movement towards anode.

Dansyl amino acid	*Standardized data (distances from origin in mm)*					*Corrected mobilities (mm)*				
	pH 2	*pH 4*	*pH 5.7*	*pH 9.4*	*pH 10.2*	*pH 2*	*pH 4*	*pH 5.7*	*pH 9.4*	*pH 10.2*
β-Alanine	17	2	−10	−7	0	23	−1.5	−18	−28	−24
Arginine	19	4	3	8	11	26.5	1.5	1.5	0	0
α-Aminobutyric acid	15	−5	−9	−6	0	20	−11.5	−17	−24.5	−24
γ-Aminobutyric acid	17	3	−9	−6	2	23	0	−17	−24.5	−20
α-Aminoisobutyric acid	16	−3	−10	−6	0	21.5	−8.5	−18	−24.5	−25
β-Aminoisobutyric acid	16	5	−9	−6	0	21.5	3	−17	−24.5	−24
Asparagine	15	−4	−8	−6	0	20	−10	−15	−24.5	−24
Aspartic acid	13	−10	−28	−21	−10	17	−20	−45	−53	−47
Azetidine-2-carboxylic acid	14	5	−11	−6	0	19	3	−20	−24.5	−24
Carnosine	17	7	3	2	3	23.5	6	1.5	−10.5	−18
Cysteic acid	2	−14	−24	−19	−13	0	−24	−38	−49	−56
Glutamic acid	14	−7	−8	−9	−9	19	−16	−15.5	−31.5	−44
Glutamine	14	1	−8	−5	0	19	−3	−15.5	−23	−22
Glycine	9	−5	−14	−5	0	11.5	−12	−24	−23	−24
Histidine	25	7	1	−1	0	35	6	−1.5	−16	−24
Homocysteine	21	3	−6	−11	−10	28	0	−12	−35	−46
Homoserine	16	0	−10	−4	0	22	−4.5	−18.5	−21	−22

4-Hydroxyproline	15	−3	−5	0	2	20	−9	−10	−14	−18
Isoleucine	16	9	−1	−1	0	21	9	−4	−16	−24
Leucine	19	10	−2	−3	2	26	10.5	−6	−19	−18
Lysine	24	8	6	2	0	33	7.5	7	−12	−24
Methionine	15	−6	−8	−2	1	19	13.5	−15	−18	−20
3-Methylhistidine	23	5	−3	−5	0	32	3	−7	−23	−24
Ornithine	24	8	6	−1	1	33	7.5	7	−16	−20
Phenylalanine	17	−2	−4	−1	0	24	−8	−8.5	−16	−24
Pipecolic acid	15	−6	−9	−4	0	19	−13.5	−17	−21.5	−22
Proline	13	−4	−11	−6	−3	17	−11	−20	−24.5	−31
Serine	14	−5	−10	−5	0	17.5	−12	−18	−23.5	−22
Taurine	2	−14	−11	−12	−6	0	−26	−20	−37	−38
Threonine	10	−4	−9	−3	1	12	−10.5	−17	−20	−20
Tryptophan	16	4	−8	−1	−1	21	1.5	−15	−16	−27
Tyrosine	17	2	−1	−1	0	23	−1.5	−4	−16	−24
Valine	15	6	−8	−5	0	20	4.5	−15	−23.5	−24
Standards										
Glucose	2	3	2	8	10.5					
K^+	67	70	69	63	54					

TABLE II

ELECTROPHORETIC MOBILITIES OF DANSYL AMINES AT pH 2 AND 4

Dansyl amine	*Standardized data (distances from origin in mm)*		*Corrected mobilities (mm)*	
	pH 2	*pH 4*	*pH 2*	*pH 4*
N-Acetyl-5-hydroxytryptamine	60	32	18	4
Agmatine	112	88	35	12
6-Aminohexanol	74	54	22	7
2-Aminoimidazole	110	51	34	7
5-Aminopentanol	79	54	24	7
Isoamylamine	80	37	24	5
Benzylamine	69	33	21	4
Isobutylamine	78	45	24	6
n-Butylamine	81	43	25	5
sec.-Butylamine	79	45	24	6
tert.-Butylamine	82	55	25	7
Cadaverine	74	0	22	–
Cystamine	86	54	26	7
1,6-Diaminohexane	88	54	27	7
1,2-Diaminopropane	96	59	29	8
1,3-Diaminopropane	88	54	27	7
Dimethylamine	82	55	25	7
Dopamine, didansyl derivative	89	44	27	6
Dopamine, tridansyl derivative	111	60	34	8
Ephedrine	70	32	21	4
Norephedrine	72	50	22	6
Epinephrine	0	0	–	–
Norepinephrine	0	0	–	–
Ethanolamine	76	54	23	7
Ethylamine	72	48	22	6
Gramine	70	41	21	5
n-Hexylamine	61	0	18	–
Histamine	93	0	28	–
Hordenine	110	76	34	10
5-Hydroxydopamine	0	0	–	–
β-Hydroxyphenylethylamine	78	51	24	6
2-Hydroxyphenylethylamine	64	0	19	–
5-Hydroxytryptamine	71	0	21	–
Metanephrine	73	0	22	–
Normetanephrine	78	0	24	–
3-Methoxy-4-hydroxy-phenylethylamine	69	0	21	–
3-Methoxy-4-hydroxy-phenylmethylamine	65	0	19	–
6-Methoxytryptamine	63	49	18	6
Methylamine	83	54	25	7
N-Methyl-3,4-dimethoxy-phenylethylamine	71	49	21	6
N-Methyldopamine	0	0	–	–
N-Methyl-4-methoxy-phenylethylamine	63	0	19	–
N-Methyl-β-phenyl-ethylamine	68	0	20	–
N-Methyltryptamine	61	0	18	–

TABLE II (*continued*)

Dansyl amine	*Standardized data (distances from origin in mm)*		*Corrected mobilities (mm)*	
	pH 2	*pH 4*	*pH 2*	*pH 4*
p-Octopamine	74	54	22	7
n-Octylamine	48	0	14	–
β-Phenylethylamine	69	0	20	–
Phenylpropanolamine	71	46	21	6
Isopropylamine	74	44	22	6
n-Propylamine	75	44	22	6
Putrescine	55	68	16	9
Spermidine	0	0	–	–
Spermine	0	0	–	–
Synephrine	0	0	–	–
Tryptamine	56	0	17	–
p-Tyramine, monodansyl derivative	76	15	23	1
p-Tyramine, didansyl derivative	106	18	32	2
Standards				
Glucose	5.5	5.5		
K^+	310*	700*		

* Calculated from position of dansylmethylamine.

TABLE III

ELECTROPHORETIC MOBILITIES OF DANSYL AMINES AT HIGHER pH VALUES

Most of the amines listed in Table II are not shown here because they were immobile at pH 5.7 and all higher pH values. Figures in brackets are the apparent R_F values, relative to glucose.

Dansyl amine	*Standardized data (distances from origin in mm)*		
	pH 5.7	*pH 9.4*	*pH 10.2*
Agmatine	19	24	15
6-Aminohexanol	7 (0.9)	8 (0.7)	11 (0.9)
2-Aminoimidazole	8 (1.0)	0	0
5-Aminopentanol	8 (1.0)	11 (0.9)	12 (1.0)
1,2-Diaminopropane	4 (0.5)	0	0
Dimethylamine	4 (0.5)	9 (0.7)	10 (0.8)
Dopamine, didansyl derivative	8 (1.0)	0	0
Ethanolamine	8 (1.0)	7 (0.6)	8 (0.7)
Ethylamine	5 (0.6)	8 (0.7)	10 (0.8)
Gramine	7 (0.9)	0	0
Hordenine	5 (0.6)	0	0
Methylamine	8 (1.0)	11 (0.9)	12 (1.0)
Phenylpropanolamine	8 (1.0)	3 (0.2)	0
Isopropylamine	8 (1.0)	4 (0.3)	0
n-Propylamine	7 (0.9)	4 (0.3)	0
p-Tyramine, monodansyl derivative	7 (0.9)	0	0
p-Tyramine, didansyl derivative	3 (0.4)	0	0

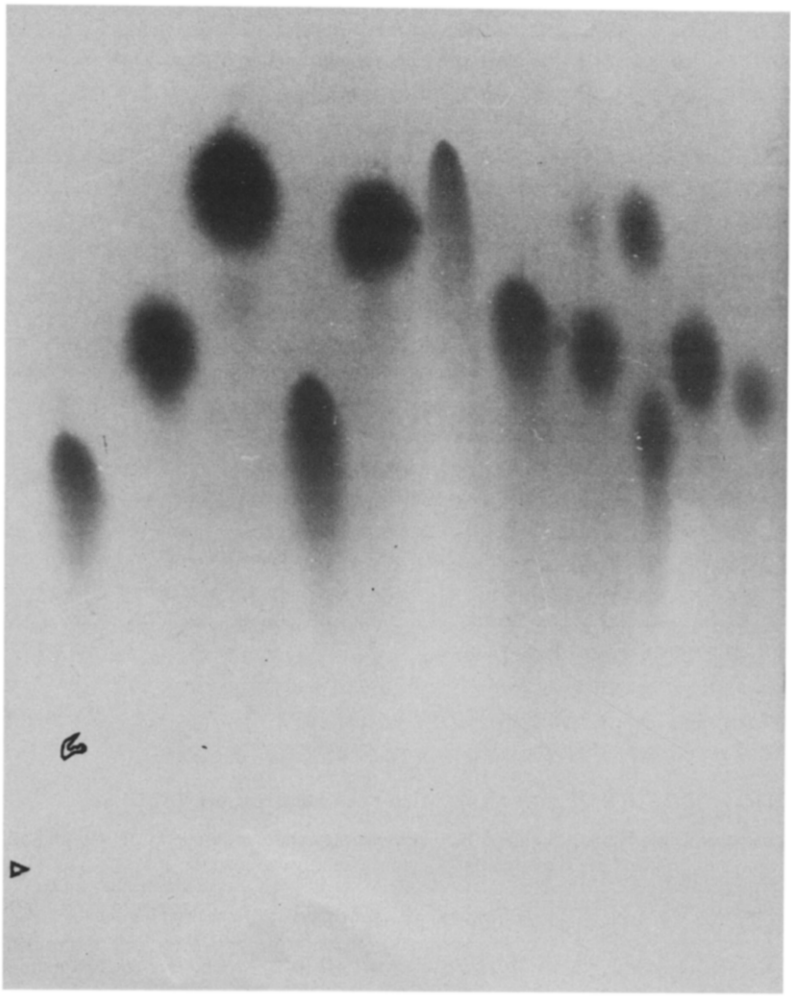

Fig. 1. Negative print of electrophoretogram run at 18 V cm^{-1} for 5.5 h. Samples, in order, from left to right, were the dansyl derivatives of tryptamine, norephedrine, methylamine, *n*-hexylamine, ethanolamine, 1,3-diaminopropane, benzylamine, valine, tyrosine (slower spot, monodansyl derivative), serine and glutamine.

water soluble to electrophorese at pH 2. The seven exceptions probably all bear more than one dansyl group. However, all dansyl amines become insoluble as the derivatising group loses its positive charge unless the molecular weight is comparatively low (*e.g.* methylamine, ethylamine) or there are other polar groups present, as in 6-amino-hexanol and agmatine. Corrected results are not given for dansyl amines which have precipitated at the origin or are moving slower than glucose since these would give the misleading impression that the sample was carrying a negative charge. The precipitation effect itself allows separation of some dansyl amines at alkaline pHs because a few are carried by the mass buffer flow and are subject to a form of adsorption chromatography.

Initial experiments at pH 2, not reported in detail here, showed that high voltage electrophoresis at 40 V cm^{-1} gave quite satisfactory results for dansyl amino acids, but caused unacceptable streaking of many dansyl amines, notably the phenylethylamines, tryptamines and polyamines. Streaking became progressively worse for all compounds as the voltage was increased to 200 V cm^{-1}. However, all derivatives shown as being mobile gave satisfactory, compact, spots under the conditions finally adopted.

Overall the results show that low-voltage electrophoresis is a useful supplementary technique for the separation of amines–amino acids, especially when the amounts available are very small. For example, electrophoresis is more efficient than thin-layer chromatography for resolving didansylputrescine/monodansyl-*p*-tyramine as well as the dansyl derivatives of ephedrine/N-methyl-4-methoxyphenylethylamine and benzylamine/phenylethylamine.

The reaction of low-molecular-weight amino and imino compounds with a higher-molecular-weight label makes the method very useful for functional group detection, since the net number of positive or negative charges carried by a monodansyl derivative at a given pH can be estimated fairly accurately. Thus a corrected mobility figure of about 20 mm corresponds to a single charge. Additional dansyl groups destroy this simple relationship: for example, they only increase mobility marginally at pH 2 because the extra charge introduced is largely balanced by an increase in molecular size.

Electrophoresis is a reliable though not quite infallible method of distinguishing between dansylated amino acids and amines: amino acids almost always show a negative charge at pH 10.2, while amines do not. Even the exceptions, amino acids containing guanidino groups, would probably arouse suspicion because they would still be soluble and mobile at pH 10.2. Moreover, electrophoresis can give indications of the presence of SH groups, unreacted NH and aromatic OH groups. This is useful because the dansylation of natural basic nitrogen fractions often gives products less completely derivatised than theoretically possible and dansyl amines bearing free phenolic hydroxy groups are sometimes isolated[11].

The dansyl chloride reaction can generate fluorescent breakdown products, monodansylated aldehydes, from lysine and ornithine[16]. However, here this has not happened as these basic amino acids have both yielded the expected didansyl derivatives, according to their corrected mobilities.

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