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Separation of 24 dansylamino acids by capillary electrophoresis with a non-ionic surfactant

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Abstract

The separation of 24 dansylamino acids was investigated by capillary electrophoresis with an additive of micelles of a non-ionic surfactant, Tween 20. Although two pairs of peaks, norvaline and methionine derivatives, and didansyltyrosine and solvent (methanol), did not show good resolution, other dansylamino acids were well separated within 70 min using 100 m*M* Tween 20 and pH 2.40. The theoretical plate numbers calculated for dansylamino acids were 28 000–111 000 with a 19-cm capillary column.

1. Introduction

Recently, capillary electrophoresis (CE) has been attracting attention for the separation of ionic analytes because it gives high theoretical plate numbers. Amino acid derivatives separated by CE or micellar electrokinetic chromatography (MEKC) include phenylthiohydantoin [1–3], dansyl [4,5], *o*-phthaldialdehyde [6,7] fluorescein isothiocyanate [7,8,9], fluorescamine [7,10] and 9-fluorenylmethyl chloroformate [7] derivatives. However, separations of twenty or more amino acids were often difficult because of insufficient resolution. We reported previously the separation of closely related peptides by using micelles of a non-ionic surfactant as an additive to buffer solution [11]. In this work, this technique was applied to the separation of 24 dansylated amino acids.

2. Experimental

Dansylamino acid derivatives were purchased from Sigma (St. Louis, MO, USA). All except glycine derivative were L-isomers. Monodansyl-L-tyrosine was purchased from ICN Biomedicals (Costa Mesa, CA, USA). Tween 20 (Fig. 1) was purchased from Wako (Osaka, Japan). These chemicals were used as received.

A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 25–50 μm I.D. and 363 μm O.D. was attached to a Spectra Chrom 200 UV detector (Spectra-Physics, San Jose, CA, USA). Detection was carried out by

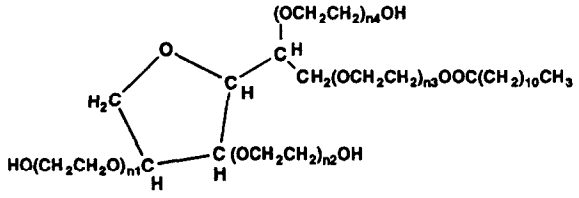

Fig. 1. Structure of Tween 20.

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on-column measurements at a wavelength of 214 nm and a detection rise time of 0.5 s through a 3-mm capillary window without polyimide. The high-voltage d.c. power supply was a Model HCZE-30PNO.25-LDSW (Matsusada Precision Devices, Kusatsu, Japan) delivering up to ±30 kV. For data recording and processing, a Chromatopac Model C-R6A (Shimadzu, Kyoto, Japan) was used. Samples were injected hydrodynamically from the anodic end of a capillary. Before each run, the column was rinsed successively with methanol, water and the separation buffer. The pH of separation solution was measured with a Beckman Φ34 pH meter calibrated at pH 4 and 7 with a commercial pH standard from Beckman.

3. Results and discussion

When Tween 20 was selected as a micellar pseudo-stationary phase to separate dansylamino acids, the most important factor was the concentration of Tween 20, which was varied from 10 to 150 m*M*. At 10 m*M*, some hydrophobic amino acid derivatives were separated but hydrophilic amino acid derivatives were largely overlapped (Fig. 2). At 150 m*M*, dansylamino acids were well separated but a long analysis time was required for the separation of 24 dansylamino acids because dansyl derivatives of hydrophobic amino acids had large capacity factors under these conditions. The concentration range of Tween 20 to achieve simultaneously optimum resolution and a reasonable analysis time was 80–100 m*M* (Fig. 3).

Although most of dansylamino acids did not change their migration order within the investigated pH range, some dansylamino acids changed their migration order depending on the pH of the buffer. The separation of glutamate, glycine, alanine and lysine derivatives was pH sensitive. As shown in Fig. 4, dansyl glutamate eluted between glycine and alanine derivatives at pH 2.19, and eluted earlier than dansylglycine at pH 2.37 or above when the concentration of Tween 20 was 100 m*M*. When the pH was raised to 2.73 in the presence of 100 m*M* Tween 20,

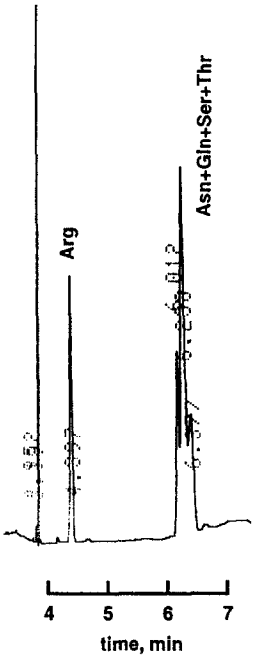

Fig. 2. Separation of dansyl derivatives of arginine, asparagine, glutamine, serine and threonine. Electrophoretic solution, 10 m*M* Tween 20 in 25 m*M* sodium phosphate buffer (pH 2.40); applied voltage, 14 kV; capillary I.D., 50 μm; column length, 35 cm (effective length, 20 cm).

dansyl glutamate and dansylglycine were well separated, but the migration times of glycine and alanine derivatives came closer.

Another pH-dependent separation was found with tryptophan, aspartate and cystine derivatives (Fig. 5). At pH 2.29 aspartate, cystine and tryptophan derivatives eluted in this order, but at pH 2.52 dansyltryptophan eluted first followed by the derivatives of aspartate and cystine.

The separation of dansylglutamine and dansylserine was achieved when the pH of separation buffer was in the range 2.33–2.73 but started to overlap at pH 2.19 and overlapped completely at pH 2.08 when the concentration of Tween 20 was set at 100 m*M*.

Dansylnorvaline and dansylmethionine were only partially resolved at pH 2.4 with 100 m*M* Tween 20, which is an optimized condition for most amino acid derivatives. The peak of didansyltyrosine overlapped with the solvent peak (methanol) caused by electroosmotic flow

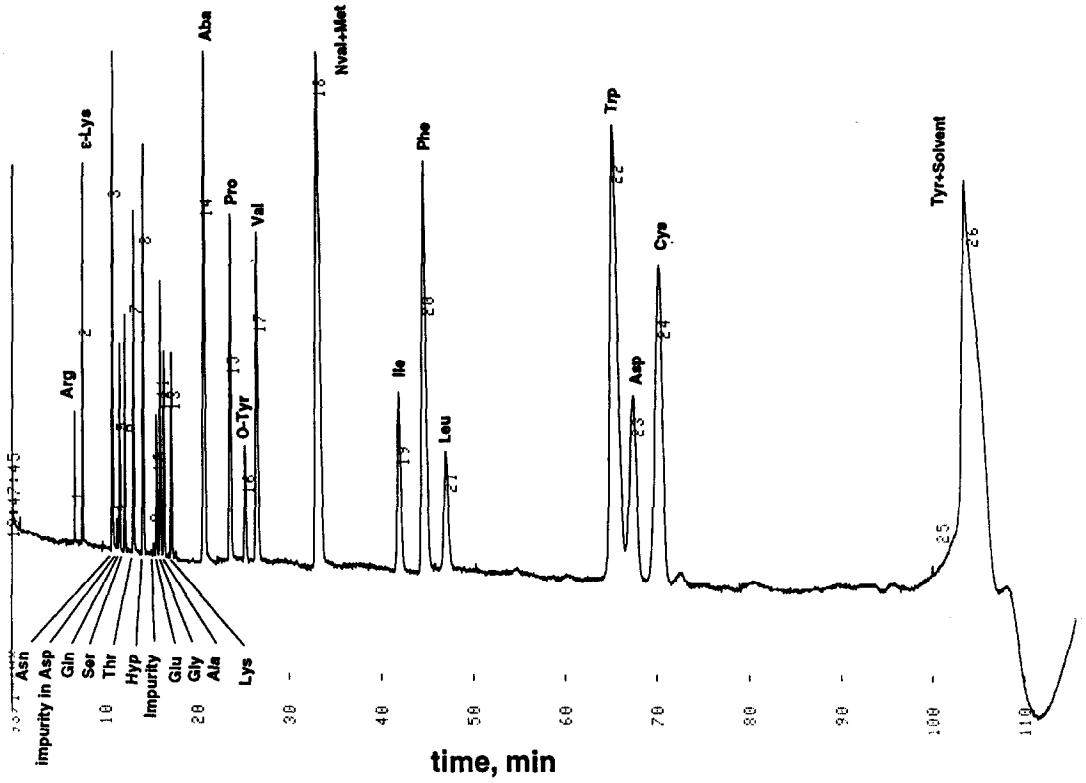

Fig. 3. Separation of 24 dansylamino acids. Dansylamino acids are represented by three-character abbreviations of the corresponding amino acids (ε-Lys is ε-dansyllysine, O-Tyr is O-dansyltyrosine, Cys is didansylcystine and Lys and Tyr are didansyl derivatives). Electrophoretic solution, 100 m*M* Tween 20 in 25 m*M* sodium phosphate buffer (pH 2.40); applied voltage, 16 kV; capillary I.D., 25 μm; column length, 34 cm (effective length, 19 cm).

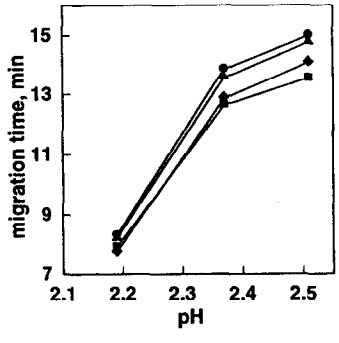

Fig. 4. Effect of pH on the separation of (■) dansyl glutamate, (◆) dansylglycine, (▲) dansylalanine and (●) didansyllysine. Electrophoretic solutions, 100 m*M* Tween 20 in 25 m*M* sodium phosphate buffer; applied voltage, 14 kV; capillary I.D., 50 μm; column length, 34 cm (effective length, 19 cm).

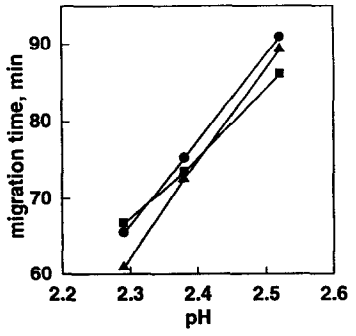

Fig. 5. Effect of pH on the separation of (▲) dansyl aspartate, (■) dansyltryptophan and (●) didansylcystine. Electrophoretic solution, 100 m*M* Tween 20 in 25 m*M* sodium phosphate buffer; applied voltage, 14 kV; capillary I.D., 30 μm; column length, 38 cm (effective length, 22 cm).

because of the slow migration of didansyltyrosine.

The migration times of dansylamino acids were plotted against the hydrophilicity values of free amino acids [12]. As shown in Fig. 6, the migration times increased with decrease in the hydrophilicity of the amino acids. In the pH range 2–3, the dimethylamino group in the dansyl group and the nitrogen atoms of the side-chain of lysine and arginine are positively charged. The carboxyl group of amino acids is hardly charged except for aspartate and glutamate, which have a second carboxyl group. As a result, the total charges of most amino acids without a charged side-chain are almost identical. Hence the migration order of dansylamino acids is regulated not by their charge but mainly by their distribution coefficients into surfactant micelles. As Tween 20 has no electric charge, the interaction is not ionic but mainly hydrophobic. The observed migration order of dansylamino acids is well explained by this hypothesis. The exceptions are the derivatives which have two dansyl groups per an amino acid (lysine, cystine and tyrosine) or those which have a negatively charged side-chain (aspartate and glutamate). Didansyltyrosine (open square in Fig. 6) eluted much more slowly than expected from the hydrophilicity value of the amino acid because of the hydrophobicity of the extra dansyl group. Didansyllysine eluted much later than ε-dansyllysine, which is also explained by the high hydrophobicity of dansyl groups. Although aspartate has a net positive charge at pH 2.4, the migration time of dansyl aspartate was much longer (open triangle in Fig. 6) than expected from its hydrophilicity value, probably owing to the negative charge of the side-chain. The side-chain of arginine not only increases the positive charge of the molecule but also enhances the hydrophilicity, which resulted in its short migration time.

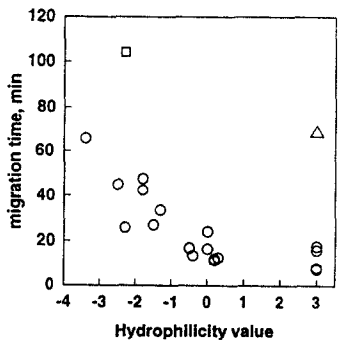

Fig. 6. Relationship between hydrophilicity values of free amino acids and migration times of corresponding dansylamino acids. △ = Dansyl aspartate; □ = didansyltyrosine. Conditions as in Fig. 3.

The migration orders observed with the dansylamino acids are also consistent with those of peptides which have a single amino acid difference [11] if we assume that the migration orders of peptides with a single amino acid difference are decided by the hydrophilicity value of the different amino acids. Human [met^{13}]-motilin eluted earlier than synthetic [leu^{13}]-motilin. Similarly, [val^{5}]-angiotensin II eluted earlier than [leu^{5}]-angiotensin II, and [sar^{1}, gly^{8}]-angiotensin II eluted earlier than [sar^{1}, ala^{8}]-angiotensin II.

4. Conclusions

If SDS is chosen as a surfactant for the separation of dansylamino acids, a problem arises from Joule heating because a relatively high concentration is required for the separation of dansylamino acids. In contrast, increasing the concentration of Tween 20 does not raise the conductivity because it is electrically neutral. The number of theoretical plate of dansylamino acids obtained with the present method (100 m*M* Tween 20 and pH 2.40), 28 000–111 000 with a 19-cm column, is comparable to that of SDS-MEKC. Although non-ionic surfactants have not yet been extensively studied as a micellar phase in CE separations, more investigations on the separation of ionic compounds can be expected.

Acknowledgements

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