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Short Communication

Studies of the electrophoretic behaviour of *p*-dihydroxyborylphenylalanine and related compounds by the three-spot method

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ABSTRACT

The effects of capillary action and electro-osmotic flow on the electrophoretic migration of *p*-dihydroxyborylphenylalanine (BPA) and related compounds were determined by spotting a sample solution at three different positions on the support. The zone electrophoretic behaviour of phenylalanine analogues was studied in support solutions of various pH values. A specific interaction between BPA and oxalate ions was found, which will be useful in the isolation of BPA from phenylalanine analogues.

INTRODUCTION

The zone electrophoretic behaviour of a compound gives useful information on its chemical state in solution. However, the observed migration distance is usually a summation of capillary action, electro-osmotic flow and electrophoretic movement [1–3]. Thus on each run it is essential to measure the factors which affect the electrophoretic mobility. Capillary action and electro osmotic flow were determined in this work by spotting a sample solution at three different positions on a support.

During studies of the physicochemical nature of BPA, which has recently been used for boron neutron capture therapy [4], a specific electrophoretic interaction was found between BPA and oxalate ions. This interaction will be useful in isolating compounds with dihydroxyboryl groups.

EXPERIMENTAL

BPA (^{10}B-enriched) was purchased from Eagle-Picher Research Laboratory (Miami, FL, USA). Amino acids of guaranteed grade were purchased from Wako (Osaka, Japan). Supporting solutions [0.05 *M* potassium oxalate (pH 1.68 at 25°C), 0.05 *M* hydrogen potassium phthalate (pH 4.01), phosphate buffer (pH 6.68), 0.01 *M* sodium borate (pH 9.18) and carbonate buffer (pH 10.02)] were also purchased from Wako. The support was Toyoroshi No. 51A filter paper (51 A, 2 × 40 cm), which was cut into pieces of 1 × 40 cm.

The apparatus and procedures used were similar to those described previously [3]. To reduce the analysis time, the electric voltage was usually applied to the spotting strips (1 × 40 cm) soon after spotting. The temperature of the migration chamber was kept constant by circulating thermostatically controlled water in a glass tube in *n*-hexane. Sodi-

um chloride solutions (0.1 *M*) were used as the electrode cell solutions. First, a spotting strip was dipped in the supporting solution and the excess of supporting solution removed by another filter paper. A 3–5 μl volume of sample solution (about $5 \cdot 10^{-3}$ *M*) was spotted at the three different positions (A, 5 cm to the cathodic side from the centre of the strip; B, the centre; C, 5 cm to the anodic side) on the strip and wetted with the support solution. Six strips (three pairs) were set in parallel in *n*-hexane in the migration chamber and the electrode cells.

A constant electric voltage was applied to two pairs for 30 min at constant temperature. One pair was allowed to stand in the migration chamber without application of the electric voltage and was used to estimate the effect of capillary action. The amino acids on the strip were detected by spraying with ninhydrin solution.

RESULTS AND DISCUSSION

The movements due to capillary action under the electrophoresis conditions used (51 A filter paper, 30 min at about 15°C) were $+0.7 \pm 0.1$, 0.0 ± 0.1 and -0.7 ± 0.1 cm at the A, B and C positions, respectively (positive movement is towards the anode, negative movement towards the cathode). The movements depended mainly on the distances from the centre of a strip and did not vary with pH. The sample spotted at the centre of the strip did not move. The relationship between the migration distance of picric acid (PA) or phenylalanine (Phe) and the voltage gradient at the three spotting positions (A, B and C) is shown in Fig. 1. The observed migration distances of PA were proportional to the voltage gradients. The movements at zero voltage gradient are due to capillary action. As the support (Toyoroshi No. 51A) contains carboxy groups which dissociate in 0.1 *M* sodium chloride solution, all the migrants were affected by an electro-osmotic flow [5].

The pK_a values of Phe were 1.83 and 9.13 [6]. Thus the small migration distances of Phe are considered to be due to its zwitterions. When discussing chemical species, capillary action and electro-osmotic flow should be taken into account. Unless stated otherwise, the following data are taken from the observed migration distances spotted at the centre of the support.

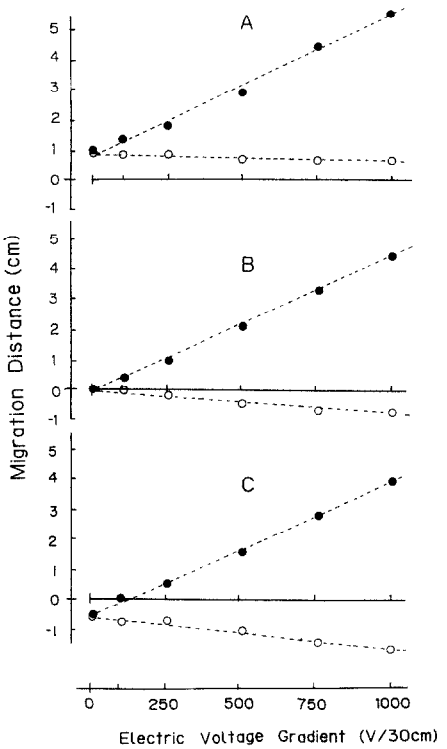

Fig. 1. Relationship between migration distance and voltage gradient. Sample solution: (●) $5 \cdot 10^{-3}$ *M* PA; (○) $5 \cdot 10^{-3}$ *M* Phe. Spotting positions: A, B and C. Electrophoresis conditions: support, Toyoroshi No. 51A; supporting solution, 0.1 *M* sodium chloride; migration temperature, about 15°C.

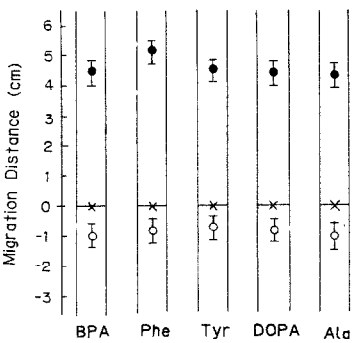

Fig. 2. Migration distances of phenylalanine analogues and picric acid in 0.1 *M* sodium chloride solution. Migrants: (●) PA; (○) sample (BPA, Phe, Tyr, DOPA and Ala). Electrophoresis conditions: 1000 V per 30 cm; time 30 min; migration temperature, 15°C; support, Toyoroshi No. 51A; starting position, ×; spotting position, B.

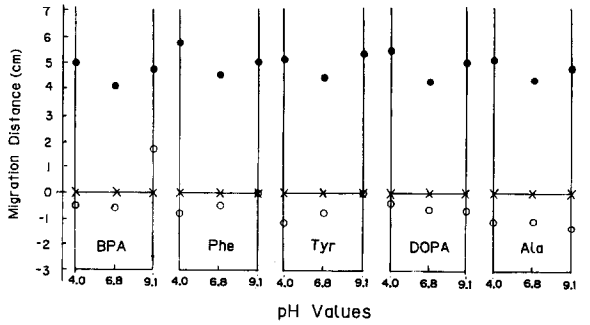

Fig. 3. Migration distances for various pH values of the supporting solution. Electrophoresis conditions and symbols as in Fig. 2. The pH values of the supporting solutions were 4.0, 6.8 and 9.1, respectively.

Fig. 2 shows the migration distances of phenylalanine analogues [BPA, Phe, tyrosine (Tyr), 3,4-dihydroxyphenylalanine (DOPA), alanine (Ala)] and PA. All the amino acids showed similar migration distances. The reproducibilities of the observed migration distances in a 0.1 *M* sodium chloride solution were not good because of its weak buffering capacity [7].

The migration distances in supporting solutions of various pH values are shown in Fig. 3. The observed migration distances of PA varied with the supporting solutions as a result of differences in the electric currents and pH values. The migration distances were similar, except for BPA at pH 9.1. The anionic migration of BPA at pH 9.1 seems to be due to the deprotonation of $-NH_3^+$ and/or the addition of OH^- to $-B(OH)_2$ groups in BPA molecules.

The electrophoresis was carried out in a strong acid solution containing oxalate ions. Surprisingly, an anionic migration of BPA was observed in the acid solution. In general, in acidic solution, the amino groups in amino acids should be protonated and the carboxy groups do not dissociate. Consequently, all the usual amino acids should behave as cations. Phe, Tyr, DOPA and Ala showed the usual cationic movements whereas BPA showed anionic movement (Fig. 4). To study this further, electrophoresis was performed in a mixture of oxalate and 0.1 *M* sodium chloride solution (Fig. 5). Results similar to those in Fig. 4 were obtained. When a hydrochloric acid solution was used instead of oxalate, this anionic movement was not observed. This means that the peculiar movement of BPA is closely related to the presence of oxalate anions. Considering the electron-deficient nature of boron atoms, it can be reasonably assumed that the dihydroxyboryl groups complex with the oxalate anions [8,9]. It was concluded that the anionic movement of BPA was due to the specific interaction between BPA and oxalate anions.

When the dissociation constant and molecular weight of the migrants are similar, it is difficult to separate them by normal electrophoresis methods.

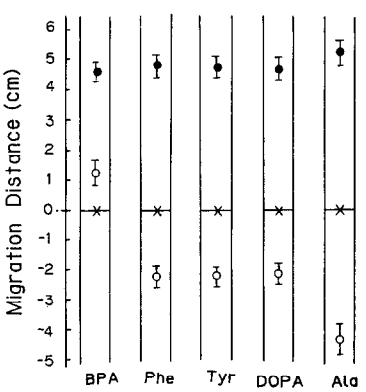

Fig. 4. Migration distances in potassium oxalate solution. Electrophoresis conditions and symbols as in Fig. 2. Supporting solution, 0.05 *M* potassium oxalate (pH 1.68 at 25°C).

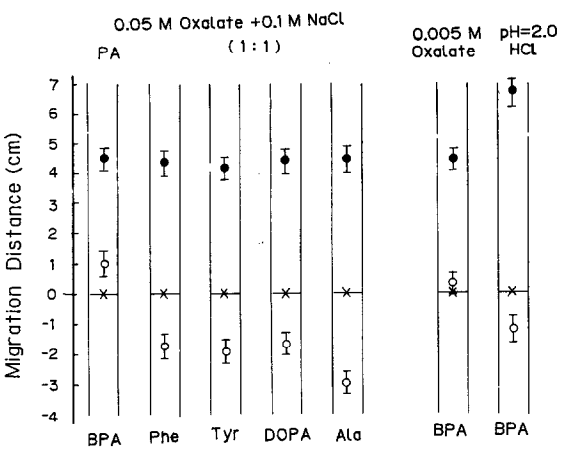

Fig. 5. Migration distances in diluted oxalate and hydrochloric acid solutions. Electrophoresis conditions and symbols as in Fig. 2. Supporting solutions: 0.05 *M* potassium oxalate–0.1 *M* sodium chloride (1:1, v/v); 0.005 *M* potassium oxalate; 0.001 *M* hydrochloric acid.

However, a dihydroxyboryl compound can easily be isolated from a mixture by using a supporting solution containing oxalate ions.

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