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Journal of Chromatography A, 738 (1996) 305–309

JOURNAL OF CHROMATOGRAPHY A

Short communication

Electrophoretic study of the interaction of *p*-boronophenylserine with carboxylic acids by the three-spot method

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Received 10 October 1995; revised 30 January 1996; accepted 30 January 1996

Abstract

The electrophoretic behaviour of *p*-dihydroxyborylphenylserine (BPS) was studied by spotting a sample solution at three different positions on the support (a pure cellulose paper). Electrophoretic migration of BPS was carried out using organic di- and tricarboxylic acid supporting solutions. A strong interaction between BPS and oxalic or citric acid was observed by paper electrophoresis. This interaction could be applied to isolation of BPS from a mixture of common amino acids. The separation of BPS from *p*-boronophenylalanine (BPA) could be made in sodium borate solution.

Keywords: Boronophenylserine; Carboxylic acids; Amino acids

1. Introduction

Many reports on the complexation with diols have been published and many kinds of physicochemical methods have been used for the confirmation of the complexation [1–3]. However, only a few papers appeared on the complexation of borate with carboxylic acids. Kustin and Pizer reported the complexation of boric acid with tartaric acid by using a temperature jump method [4]. Duin et al. studied the interaction of boric acid by using ^{11}B spectroscopy [5]. Recently, Singhal et al. reported new ligands for boronate affinity chromatography [6].

In a previous paper [7], we reported the electrophoretic behaviour of *p*-boronophenylalanine (BPA) and related compounds by the three-spot method. A specific interaction between BPA and oxalate ions was found which could be used for the specific separation of BPA. We continued to investigate the interaction of BPA with other carboxylic acids and found that the interaction largely depended upon the electrostatic and stereochemical structure of the carboxylic acids [8,9]. The infrared spectral behaviour was fully consistent with the migration behaviour in the three-spot zone electrophoresis [10].

BPA is a famous compound which has been actually used for reactor therapy. To improve the solubility of BPA, we introduced an –OH moiety into the β-carbon atom of BPA resulting in BPS [11]. On studying the charge state of BPS in solution, we found a strong interaction between oxalic or citric acid supporting solution. The differ-

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0021-9673/96/$15.00
PII S0021-9673(96)00116-1

ence between BPS and BPA is also reflected in their electrophoretical behaviour using alkaline borate solution.

2. Experimental

BPS was newly synthesized by reacting *p*-boronophenylaldehyde with ethyl isocyanoacetate [11]. BPA was purchased from Eagle-Picher (Miami, FL, USA). Malonic, tartaric and citric acid, and buffer solutions [0.05 *M* potassium oxalate (pH 1.68 at 25°C), 0.05 *M* potassium hydrogenphthalate (pH 4.01), phosphate buffer (pH 6.68), 0.01 *M* sodium borate (pH 9.18)] were purchased from Wako (Osaka, Japan).

The three-spot method is a type of zone electrophoresis. The effect of capillary action and electroosmotic flow on electrophoretic migration of a sample and standard solutions under a given condition can be simultaneously measured by this method. The usual protocol requires one to wait for a certain time in which an equilibrium should be attained in a migration chamber. However, this waiting time for equilibration is not desirable from the viewpoint of saving time. On our apparatus, we cannot spot a sample solution on a support after immersing the support in *n*-hexane, which is used to prevent vaporization of a supporting solution from the surface of the support. Usually, we set a spotting strip in a migration chamber soon after spotting a sample solution on the strip. Although the total time for electrophoresis is shortened in our procedure, it is necessary to estimate the effect of capillary action, which is measured by spotting a sample solution at three different positions.

Apparatus and procedures were similar to those described previously [7]. A 5-μl volume of sample solution or standard solution ($5 \cdot 10^{-2}$ *M*) was spotted respectively at 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 a filter paper (Toyoroshi No. 51A, pure cellulose paper) wetted with a supporting solution. Six spotting strips (1×40 cm, three pairs) were set in parallel in *n*-hexane in the migration chamber and the electrode cells. Unless stated otherwise, a 0.1 *M* NaCl solution was used as the electrode cell solutions. A constant electric voltage was applied to two pairs for 30 min at constant temperature. One pair was allowed to stand in the chamber without application of electric voltage. BPS on the strip was detected by spraying with ninhydrin solution.

3. Results and discussion

In order to investigate the charge state and interaction of BPS in solution, we carried out three-spot zone electrophoresis using various supporting solutions (see Fig. 1)

The observed migration distance is a summation of capillary action, electroosmotic flow and electrophoretic movement. Thus, on each run, we measured capillary action by allowing a strip spotted at the three positions (A, B, C) to stand without electric voltage. The positive movement is towards the anode and negative movement towards the cathode. The average movements due to capillary action were $+0.7 \pm 0.2$, 0.0 ± 0.2, -0.7 ± 0.2 cm at the A, B and C spotting positions under the electrophoresis conditions (Toyoroshi No. 51 A filter paper, 30 min at about 20°C), respectively. Generally speaking, the movement due to capillary action depends mainly on the distance from the centre of a strip and so does not vary with pH. Thus, a sample spotted at the centre does not move by capillary action under ideal conditions. The movement of neutral molecules spotted at the centre during electrophoresis is due to an electroosmotic flow which should depend on the number and degree of dissociation of carboxyl groups present as an impurity in the cellulose paper. To keep the pH of a supporting solution constant during electrophoresis, it is desirable to use a solution having large buffering capacity as a supporting solution. In the usual separation experiment, we used a commercially available buffer solution as a supporting solution. It seemed that the pHs of oxalate, phthalate and phosphate buffer supporting solution after electrophoresis did not greatly differ from the initial pH. In acidic and near neutral regions, usual amino acids should show a cation or zwitterion movement depending on the dissociation of the carboxyl moiety of the amino acid. We observed short migration distances of BPS on the cation side of the strip when we spotted BPS at the centre of the

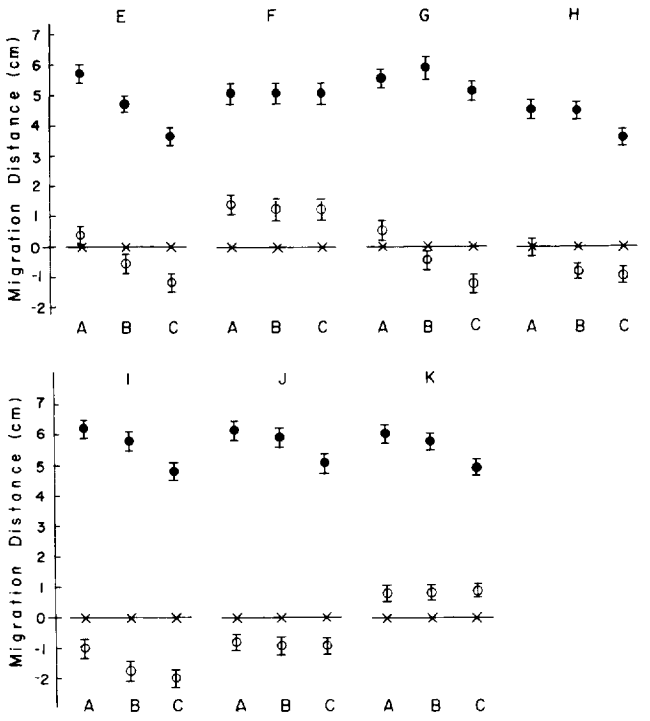

Fig. 1. Observed migration distances of *p*-boronophenylalanine in various supporting solutions. Migrants: (●) picric acid (PA); (○) BPS. Electrophoresis conditions: 1000 V/30 cm; migration time, 30 min; migration temperature, 20°C; support, Toyoroshi No. 51A; starting position, x; spotting positions: A, 5 cm to the cathodic side from the centre of the strip; B, the centre; C, 5 cm to the anodic side; Electrode cell solution: 0.1 *M* NaCl. Supporting solutions: E, 0.1 *M* NaCl; F, oxalate buffer; G, phthalate buffer; H, phosphate buffer; I, 0.1 *M* malonic acid; J, 0.1 *M* tartaric acid; K, 0.1 *M* citric acid.

strip and carried out the electrophoresis using 0.1 *M* NaCl (weak acidic), phthalate (pH 4.0) and phosphate (pH 6.8) supporting solutions, respectively. This movement is mainly due to electroosmotic flow because BPS exists mostly as zwitterions (non-movement ion) over these pH regions. When we carried out the electrophoresis using oxalic acid supporting solution, we observed unusual anionic movement which was due to a complexation of BPS with oxalate. This complexation seemed to be similar to those of BPA with organic carboxylic acids [7]. Using 0.1 *M* malonic, tartaric or citric acid supporting solution, we further carried out the electrophoresis of BPS. Cationic movement of BPS was observed on the malonic and tartaric acid supporting solution while anionic movement was observed on the citric acid solution. The observed migration distance of BPS was longer in malonic acid than in tartaric acid. Thus, according to the observed migration distances and migration direction, the degree of complexation of BPS seemed to increase in the order malonic acid<tartaric acid<oxalic and citric acids.

Recently, we use 0.1 *M* NaCl solution as electrode cell solutions to save the reagent for the supporting solution and electrode cell solution. To test the limit of 0.1 *M* NaCl solution usage, we carried out the electrophoresis for more than 30 min. In 45 min and 60 min migration, the migration distances of BPS on 0.1 *M* NaCl electrode cell solutions differed largely from those of 0.1 *M* tartaric acid electrode cell solutions. In the case without supply of tartaric acid from the electrode cell, the pH on the support after more than 30 min migration would be shifted to the alkaline side. This means that the 0.1 *M* NaCl electrode cell solution cannot be used for migration times longer than 30 min at the voltage gradient of

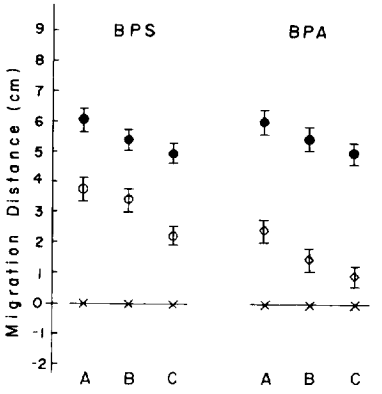

Fig. 2. Observed migration distances of *p*-boronophenylserine and *p*-boronophenylalanine in sodium borate supporting solution. Migrants: (●) picric acid (PA); (○) BPS; (◇) BPA. Electrophoresis conditions as in Fig. 1. Supporting solution, 0.01 *M* sodium borate buffer (pH 9.18).

1000 V per 30 cm. For long migration times, we should employ the same electrode cell solution as the supporting solution (Kitaoka, unpublished data).

Many biomolecules have been separated using affinity chromatograpy based on the interaction of the hydroxyboryl group with *cis*-diols [6]. Previously, we proposed the application of a specific interaction to zone electrophoresis, in which we suggested using a supporting solution containing a reagent complexing with a migrant. Electrophoretically, BPS will be separated from an amino acid having no dihydroxyboryl moiety by using an oxalic or citric acid supporting solution. However, BPS and BPA will not be separated by this solution. Using sodium borate supporting solution, we carried out the electrophoresis of BPS and BPA (see Fig. 2 and Table 1). BPS migrated twice as far as BPA. As the molecular mass of BPS is nearly equal to that of BPA, this higher migration of BPS seems to be due to the deprotonation of the $-NH_3^+$ group of BPS which occurred at a lower pH than for BPA. The separation of BPS from BPA could be made by using this alkaline borate solution.

Generally, on electrophoretical separation of equimolecular mass compounds, the application of a complex formation reaction or a pH change of the supporting solution should be considered.

Table 1
Observed migration distances of *p*-boronophenylserine (BPS), *p*-boronophenylalanine (BPA) and picric acid (PA)

Migrants	Supporting solution	Observed migration distances (cm)		
		A[a]	B	C
BPS	0.1 *M* NaCl	0.4±0.2	−0.5±0.2	−1.2±0.2
PA	0.1 *M* NaCl	5.7±0.2	4.8±0.2	3.6±0.2
BPS	Oxalate buffer	1.4±0.2	1.2±0.2	1.2±0.2
PA	Oxalate buffer	5.0±0.2	5.0±0.2	5.0±0.2
BPS	Phthalate buffer	0.5±0.2	−0.4±0.2	−1.2±0.2
PA	Phthalate buffer	5.6±0.2	5.8±0.2	5.1±0.2
BPS	Phosphate buffer	0.0±0.2	−0.7±0.2	−0.9±0.2
PA	Phosphate buffer	4.5±0.2	4.5±0.2	3.5±0.2
BPS	0.1 *M* Malonic acid	−1.0±0.2	−1.7±0.2	−2.0±0.2
PA	0.1 *M* Malonic acid	6.3±0.2	5.8±0.2	4.8±0.2
BPS	0.1 *M* Tartaric acid	−0.8±0.2	−0.9±0.2	−0.9±0.2
PA	0.1 *M* Tartaric acid	6.1±0.2	5.9±0.2	5.0±0.2
BPS	0.1 *M* Citric acid	0.8±0.2	0.8±0.2	0.9±0.2
PA	0.1 *M* Citric acid	6.0±0.2	5.8±0.2	4.9±0.2
BPS	Borate buffer	3.7±0.2	3.4±0.2	2.2±0.2
PA	Borate buffer	6.0±0.2	5.4±0.2	4.9±0.2
BPA	Borate buffer	2.4±0.2	1.4±0.2	1.0±0.2
PA	Borate buffer	6.0±0.2	5.5±0.2	5.0±0.2

Electrophoresis conditions: support, Toyoroshi No. 51A; 1000 V/30 cm; 30 min; temperature ca. 20°C.
[a] Spotting positions: A, 5 cm to the anodic side; B, the centre; C, 5 cm to the cathodic side. Positive movement is towards the anode, negative movement towards the cathode.

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