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Short communication

Determination of optical purity of phosphonic acid analogues of aromatic amino acids by capillary electrophoresis with α-cyclodextrin

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Abstract

A simple and efficient method for the determination of enantiomeric purity of structurally diverse phosphonic and phosphinic acid analogues of phenylalanine and phenylglycine using capillary electrophoresis is presented. These preliminary studies indicated that the enantiomer separation is strongly dependent on the structure of the aminophosphonic acid.

Keywords: Enantiomer separation; Phosphonic acids; Amino acids; Phosphinic acids

1. Introduction

Aminophosphonic acids are a class of structural analogues of amino acids obtained by replacement of amino acid carboxylic group by phosphonic or phosphinic acid moiety. This class of compounds exhibits a wide range of promising biological activities, with their commercial importance ranging from medicine to agriculture [1]. Most of the observed activities usually derive from the competition of aminophosphonic acid with amino acid for the active centre of an enzyme or other cellular receptor. In most cases, only one of the enantiomers is active and thus the availability of simple and efficient methods for the determination of optical purity of aminophosphonic acids is indispensable. Usually, the optical purity of these acids has been established by comparison of specific rotation with the highest value given in the literature. This method applies, however, only to known compounds. In a limited number of cases the enantiomeric purity of aminophosphonic acids has been proved using NMR or chromatography and usually requires derivatisation of these acids prior to analysis [2–7]. The only methods for direct determination of enantiomeric composition aminophosphonic acids include: formation of chiral complexes of these acids with palladium(II) ions and their analysis by ^{31}P NMR [8], HPLC separation using stationary chiral phases [9], and the use of capillary electrophoresis [10]. Although, the latter method seems to be most promising, available reports consider rather specific applications and its standard use requires more detailed studies. In this paper we report preliminary studies on the use of this technique to determine the enantiomeric composition of aminophosphonic acids

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obtained by various preparative methods. Regarding the possibility of UV detection, we limited our studies to analogues of aromatic amino acids.

2. Experimental

2.1. Chemicals

Aminophosphonic acids, in both racemic and optically active forms, were either available from previous studies [11–13] or were obtained by standard method [14,15]. The water used was purified with a Milli-Q system (Milipore, Bedford, MA, USA).

2.2. Apparatus, background electrolyte and experimental procedure

Electrophoretic experiments were performed on P/ACE 5000 capillary electrophoresis system (Beckman, Palo Alto, CA, USA). Capillary zone electrophoresis was performed in a fused-silica capillary 57 cm (50 cm to the detector)×75 μm. I.D.×375 μm. O.D. obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary was cooled with fluorocarbon liquid and the temperature was set at 25±0.1°C. UV detection was used with a deuterium lamp operated at 214 nm (bandpass filter).

To obtain a required background electrolyte (BGE) solution, an appropriate amount of α-cyclodextrin to reach final concentration of 40 m*M* was dissolved in 0.1 *M* phosphate buffer adjusted to pH 7.5 with 0.1 *M* NaOH (one or two drops per 100 ml). Experimental solutions were prepared by dissolving appropriate amounts of aminophosphonic acid in water in order to obtain its 1 m*M* final concentration. Experimental procedures were identical to those described earlier [16] and were as follows: at the start and end of each working day, the capillary was washed with 0.1 *M* NaOH solution (40 min) and water (2 min). Prior to every run, the capillary was washed with 0.1 *M* NaOH (2 min), water (2 min) and BGE (1 min). The sample was injected hydrostatically (3 s duration) by applying pressurized nitrogen. The electrophoretic separation was performed at applied voltage 20 kV.

3. Results and discussion

Preliminary experiments concerning appropriate pH buffer selection showed that the optimal conditions for all further experiments are 0.1 *M* phosphate buffer adjusted to pH=7.5. At this pH the electrophoretic mobility of the examined compounds was maximal with applied voltage set to 20 kV and sample injection time 3 s.

The typical way of representing the enantiomers separation in capillary electrophoresis is the separation selectivity (α) and resolution (R_S) and can be expressed by following equations:

$$\alpha = \frac{\mu_1}{\mu_2} \tag{1}$$

$$R_S = \tfrac{1}{4} \cdot N^{1/2}(\Delta\mu/\mu_{av}) \tag{2}$$

where, μ_1 and μ_2 are the electrophoretic mobility of each enantiomers, N is the theoretical plates number, $\Delta\mu$ is a mobility difference between enantiomers and μ_{av} is average mobility. The obtained results for ten enantiomer pairs separated with α-cyclodextrin are presented in Table 1.

The presented studies indicated the usefulness but in some extend also showed the limitations of the use of α-cyclodextrin enhanced capillary electrophoresis for the determination of optical purity of aminophosphonic and aminophosphinic acids. As seen from Table 1, when using racemic mixtures of these acids only 10 out of 20 compounds (presented at the Fig. 1) were separated. From the Table 1 it can also be evaluated that the separation selectivity necessary for the baseline separation is 1.02 and higher or R_S higher or close to 2. Some representative cases are shown in Fig. 2. Those differences in degree of enantiomers separation are caused by the nature of α-cyclodextrin used as discrimination factor and by the properties of the structure of the examined compounds. A separation mechanism based on inclusion–complexation of compounds to be separated with α-cyclodextrin was employed. It should be similar for all aromatic aminophosphonic acids, where hydrophobic interactions of aromatic ring with cyclodextrin are especially important. Although, using our data it is difficult to draw meaningful relationship between the structure of aminophos-

Table 1
Separation of racemic mixtures compounds 1–10 in the presence of α-cyclodextrin. BGE: 40 m*M* α-cyclodextrin in 0.1 *M* phosphate buffer, pH 7.5, 20 kV, 3 s injection time

Compound number	t_1 (min)	t_2 (min)	$\mu_1 \cdot 10^{-6}$ ($cm^2\ V^{-1}\ s^{-1}$)	$\mu_2 \cdot 10^{-6}$ ($cm^2\ V^{-1}\ s^{-1}$)	α	R_S
1	17.06	18.70	222.74	203.21	1.096	8.81
2	16.70	16.88	227.54	225.12	1.010	0.58
3	17.68	18.26	214.93	208.11	1.032	2.78
4	17.08	17.35	222.48	219.02	1.015	1.35
5	14.89	15.05	255.20	252.49	1.010	1.12
6	14.92	15.36	254.69	247.40	1.029	2.02
7	15.43	15.51	246.27	245.00	1.005	–
8	17.62	17.86	215.66	212.77	1.013	0.75
9	18.17	18.56	209.14	204.74	1.021	1.94
10	15.06	15.19	252.32	250.16	1.008	0.75

phonic acid and the separation efficiency, there is a possibility to make some general remarks. As expected, the separation of enantiomers is strongly depended on the substitution of aromatic ring. For example, it is not surprising that analogues of phenylglycine bearing hydrophilic hydroxy-, methoxy- or amino- substituents (compounds **14**, **16**, **17**) were not separated while those bearing halogen (compounds **3**–**8**) atoms were. However, it seems not to be a main cause of lack of their separation because we were not able to separate all the used phosphonic acid analogues of phenylglycine substituted in *para* position, despite of the chemical character of substituent. Moreover, as shown by preliminary studies replacement of α-cyclodextrin by β-cyclodextrin offers a possibility of separation of these acids. Similarly as in previous paper [7], phosphonic acid analogues were separated more efficiently than their phosphinic acid counterparts. This is because aminophosphonic acids deliver one more hydroxylic group (fully deprotonated at pH 7.5) than the corresponding phosphinic acids. This leads to the increased hydrophilic interactions with hydroxyl groups of cyclodextrin and increased stability of its complexes with aminophosphonic acids resulting in longer migration times, which in turn has a positive effect on enantioselectivity.

The usefulness of α-cyclodextin based resolution of aminophosphonic acids by means of capillary electrophoresis was shown in determination of optical purity of phosphonic acid analogues of dihydroxyphenylalanine (compound **1** in Fig. 1) and of phenylglycine (compound **2**). As shown in Fig. 3a isomer of compound **1** of R configuration, obtained by resolution of its diethyl ester with dibenzoyl-L-tartaric acid [11] contains 98% of isomer R, and is contaminated with minute quantities of additional compounds. S isomer of compound **2** (Fig. 3b) obtained by resolution of mixed complex with threonine and Cu^{2+} [12] exhibits only 10% enantiomeric excess determined by CE. On the contrary, the specific rotation measurement ($-5°$) suggests 25% enantiomeric excess. This shows that the specific rotation is not suitable for the evaluation of the enantiomeric purity and the capillary electrophoresis is in superior in those types of tests. This limited set of data indicates the potency of cyclodextrin-based capillary electrophoresis as a tool for the determination of chemical, as well as optical purity of aminophosphonic acids.

4. Conclusions

This work presents potential method for separation and determination of optical purity of enantiomers of phosphonic acid analogues of aromatic amino acids by means of capillary electrophoresis, using simple conditions of analysis such as underivatized aminophosphonic acids separated in phosphate buffer with α-cyclodextrin and direct UV detection.

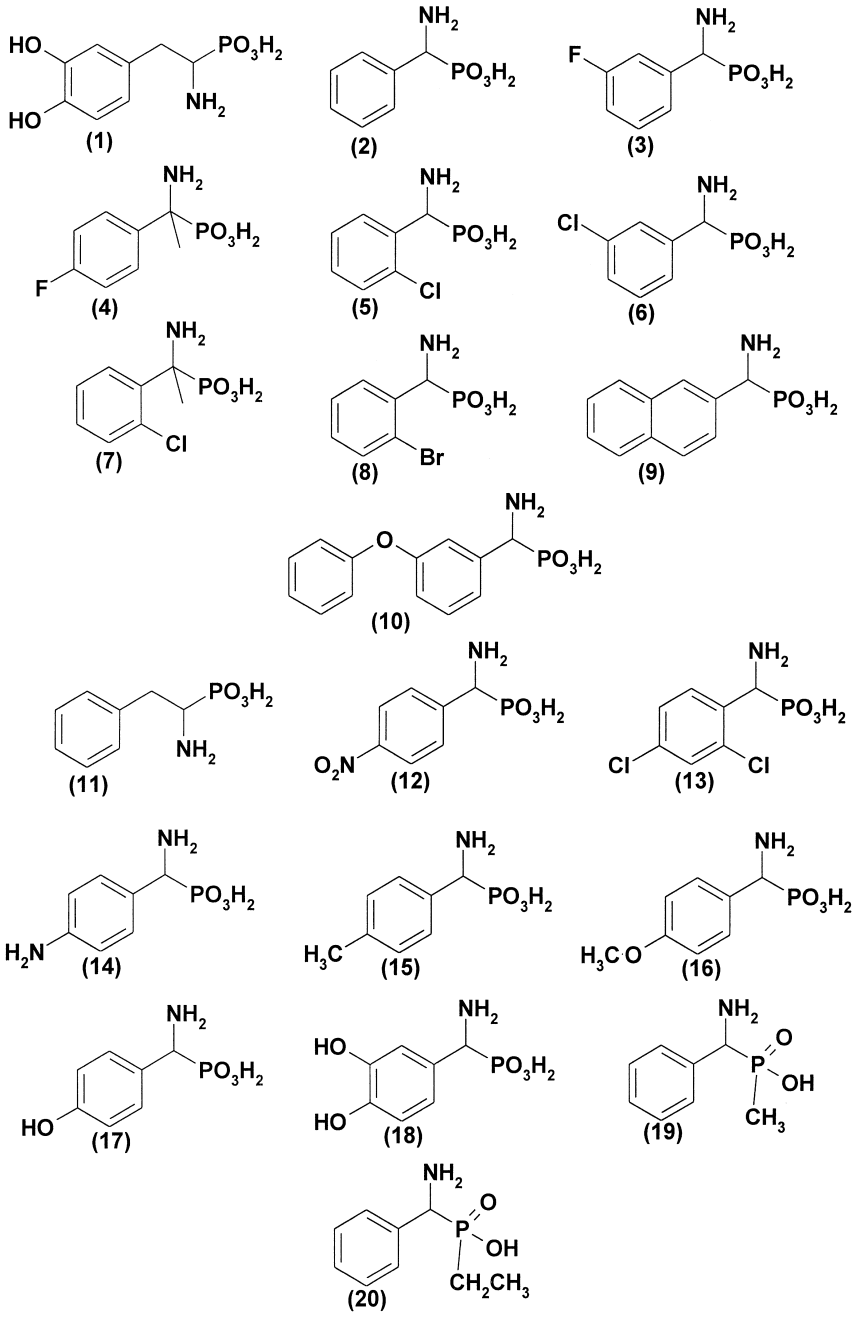

Fig. 1. Chemical structures of phosphonic and phosphinic acid analogues of aromatic amino acids. Compounds **1**–**10** undergoing separation; **11**–**20** not separated.

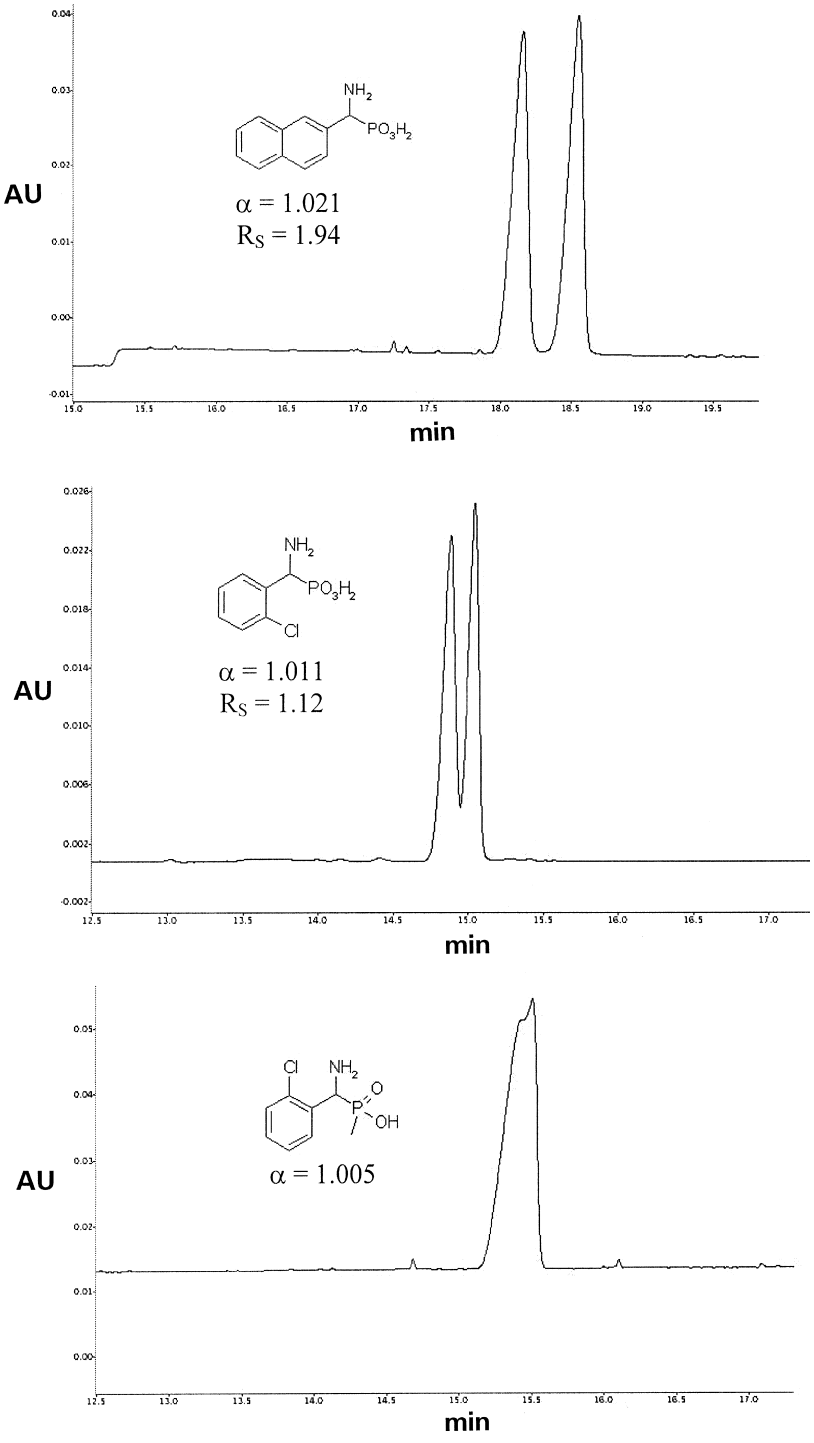

Fig. 2. Representative examples of separation of racemic aminophosphonic acids. BGE: 40 m*M* α-cyclodextrin in 0.1 *M* phosphate buffer, pH 7.5, 20 kV, 3 s injection time.

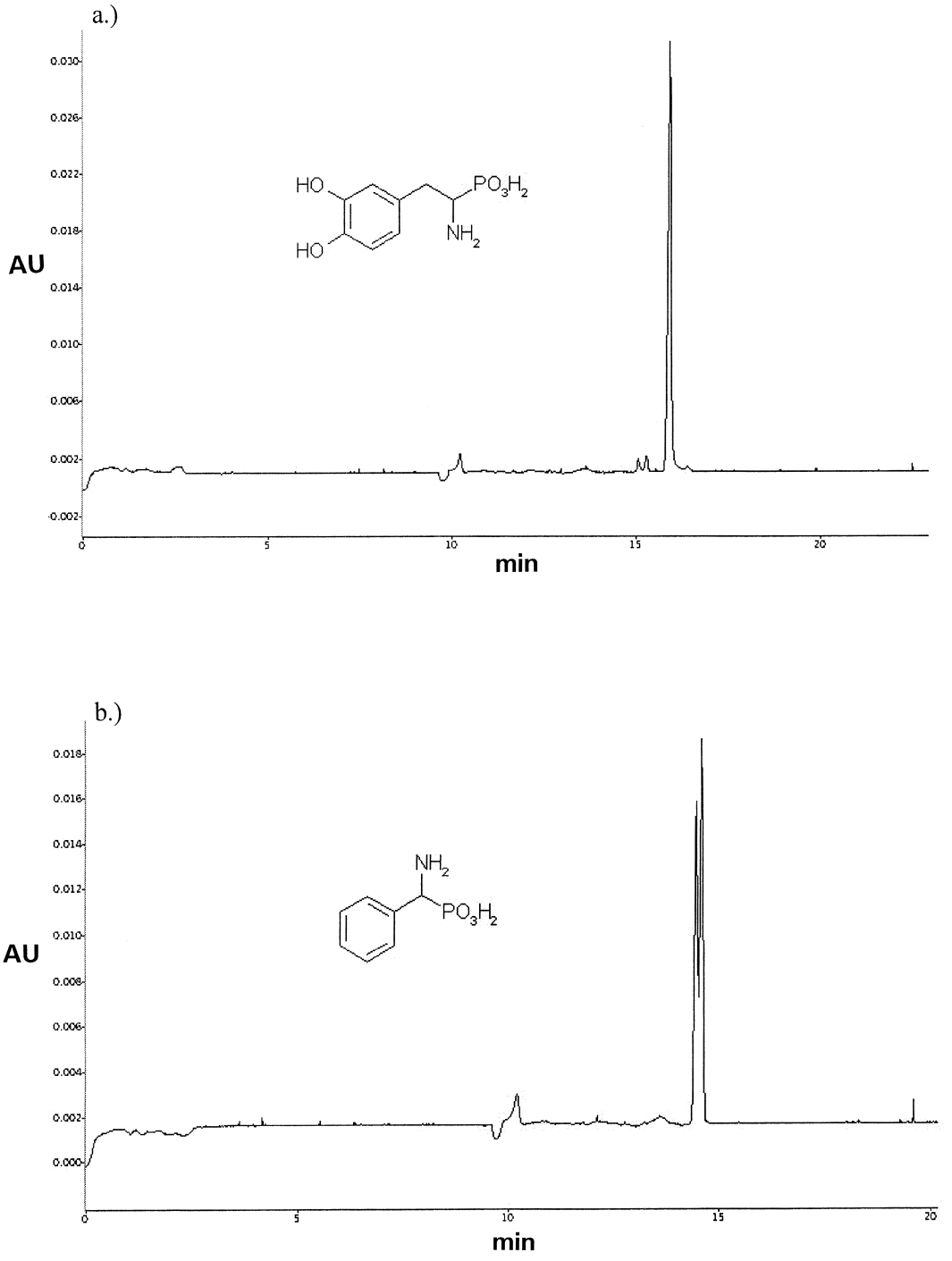

Fig. 3. Determination of optical purity (a) of (R)-1-amino-2-(3,4-dihydroxypenyl)ethanephosphonic acid (compound **1**) of specific rotation of −53° (c=1, 5 *M* HCl) and (b) (S)- aminobenyzylphosphonic acid (compound **2**) of specific rotation −5° (c=1, 1 *M* NaOH). BGE: 40 m*M* α-cyclodextrin in 0.1 *M* phosphate buffer, pH 7.5, 20 kV (reverse polarity), 3 s injection time.

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