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Ion-exchange high-performance liquid chromatography of diastereoisomers of some phosphonodipeptides

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ABSTRACT

The chromatographic behaviour of L-L,D-peptides Val–AlaP, Leu–PheP, Ala–AlaP, Phe–LeuP, Leu–PheP(O-iso-C_3H_7)$_2$ and Leu–Phe on aminopropyl and sulphonyl sorbents was studied. An increase in the H_3PO_4 concentration in the eluent resulted in a decrease in the retention but hardly affected the selectivity of the separation of the phosphonopeptides on an aminopropyl sorbent. The retention and selectivity of the separation of the phosphonopeptides on a sulphophenyl sorbent decreased with increasing H_3PO_4 concentration in the eluent. The optimum conditions for the separation of the diastereoisomers were found.

INTRODUCTION

Aminophosphonic acids[a] and phosphonopeptides (PPepts) are a promising class of bioactive compounds [1]. The separation of diastereoisomers of phosphonodipeptides (PDs) that differ in the configuration of the α-carbon atom of the aminophosphonic acid residues by normal and reversed-phase (RP) high-performance liquid chromatography (HPLC) was described in a previous paper [2]. It should be noted that the separation of hydrophilic and poorly retained PDs by RP-HPLC is sometimes difficult. Hence it seemed expedient to use ion-exchange HPLC (HPIEC) for this purpose. Only a few data concerning the separation of PDs by IEC have been reported previously. The separation of diethyl esters of 1-(N-L-alanylamino)benzylphosphonic acid isomers by IEC has been described [3].

The aim of this work was to determine the optimum conditions for the HPIEC of PD on aminopropyl and sulphophenyl sorbents.

[a] Generally accepted abbreviations for α-aminophosphonic acids were used, *e.g.*, L,D-1-(N-L-alanylamino)ethylphosphonic acid = L-Ala-L,D-AlaP.

EXPERIMENTAL

Chromatographic conditions

The experiments were performed on an LKB (Bromma, Sweden) liquid chromatographic system consisting of a Model 2150 HPLC pump, a Model 7410 injector, a Model 2140 rapid spectral detector set at 225 nm and a Model 220 recording intergrator. The column used were (1) Separon SIX NH_2, 5 μm (150 × 3.3 mm I.D.) from Tessek (Prague, Czechoslovakia) and (2) Nucleosil 100 5-SA, 5 μm (200 × 4.0 mm I.D.) from Macherey–Nagel, (Düren, Germany). The mobile phases were methanol (0–5%)–orthophosphoric acid (0.1 m*M*–0.5 *M*) with isocratic elution. The flow-rate was (1) 0.25 or (2) 0.4 ml/min.

Materials

L-L,D-Dipeptides Val–AlaP (**1**), Leu–PheP (**2**), Ala–AlaP (**3**), Phe–LeuP (**4**), Leu–PheP(O-iso-C_3H_7)$_2$ (**5**) and Leu–Phe (**6**) were obtained as described [4,5]. Orthophosphoric acid and methanol were used as received. Water was doubly distilled and filtered for HPLC use.

RESULTS AND DISCUSSION

When PPepts are chromatographed on an aminopropyl sorbent with an eluent containing acids a mode of anion-exchange chromatography is realized. An increase in the phosphoric acid concentration in the mobile phase results in a decrease in the PPept retention but has virtually no effect on the selectivity of separation of diastereoisomers (Fig. 1). The retentions of the PDs (**1**–**4**) increase in the order **3** > **1** > **2** > **4**. Hence we can conclude that under the conditions used hydrophobic interactions between PPepts and alkyl fragments of the sorbent have a moderate effect on their retention. Both changes in the amino acid sequence (compounds **2** and **4** and the volume of the substituent in the α-position in the aminocarboxylic acid residue (compounds **1** and **3** exert a weak effect on the retention and selectivity. The selectivity of PDs separation ranges from 1.2 to 1.6 depending on their structures (Fig. 1). The L–L isomers have lower retentions. Eluents containing 0.1–10 m*M* orthophosphoric acid and 5% methanol are the optimum for the separation of PDs on an aminopropyl sorbent (Fig. 2). In the absence of methanol in the eluent the efficiency of separation of the PDs decreases.

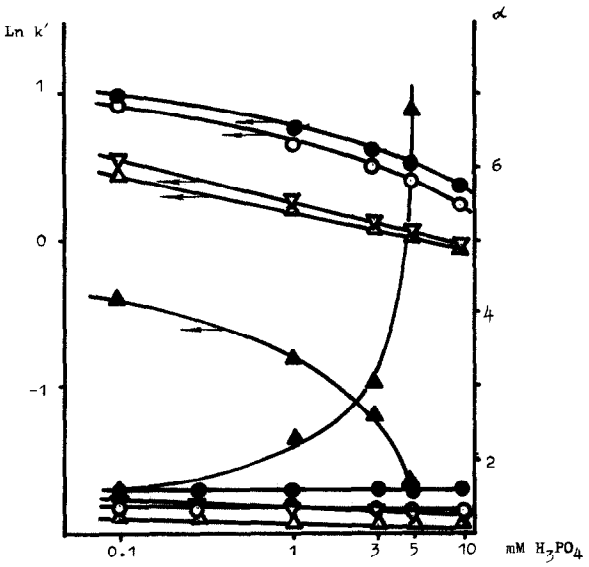

Fig. 1. Effect of orthophosphoric acid concentration on logarithm of the capacity factors (ln *k'*) of L–D isomers and the selectivity (α) of diastereoisomer separation. Column, Separon SIX NH_2, 5 μm (150 × 3.3 mm I.D.); eluent, H_3PO_4–methanol (95:5); flow-rate, 0.25 ml/min. Compounds: ● = L-Phe–D-LeuP (**4**); ○ = L-Leu–D-PheP (**2**); ▽ = L-Val–D-AlaP (**1**); △ = L-Ala–D-AlaP (**3**); ▲ = L-Leu–D-Phe (**6**).

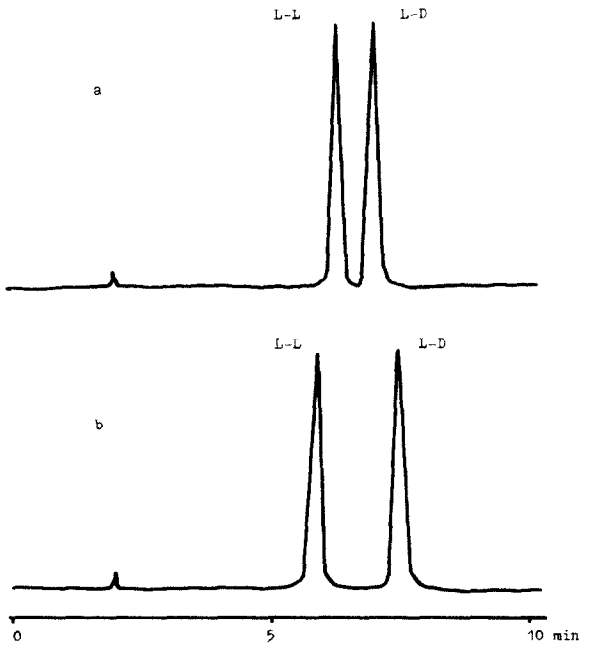

Fig. 2. Chromatograms of the separation of PPepts diastereomers. Column, Separon SIX NH_2, 5 μm (150 × 3.3 mm I.D.); eluent, 0.1 m*M* H_3PO_4–methanol (95:5); flow-rate, 0.25 ml/min. (a) L-Ala–L,D-AlaP (**3**); (b) L-Val–L,D-AlaP (**1**).

It is interesting to determine the effect of replacement of a carboxylic group by a phosphonic group on the chromatographic behaviour of peptides. For an aminopropyl sorbent the effect of the concentration of orthophosphoric acid in the eluent on the retention and selectivity of separation of the diastereoisomers of the carboxylic peptide **6** differs greatly from that for the phosphono analogues **2** (Fig. 1). The sharp increase in the selectivity of separation of diastereoisomer with increase in the acid concentration in the eluent can be explained by the difference in the p*K* values of carboxylic groups of L–L and L–D peptides. It has been shown that under conditions of RP-HPLC the selectivity of separation of diastereoisomers changes appreciably just within the range of pH values of the eluent where the peptides are converted from the ionized to the molecular form [4].

The retention of PPepts **2** and **4** on the phenyl-

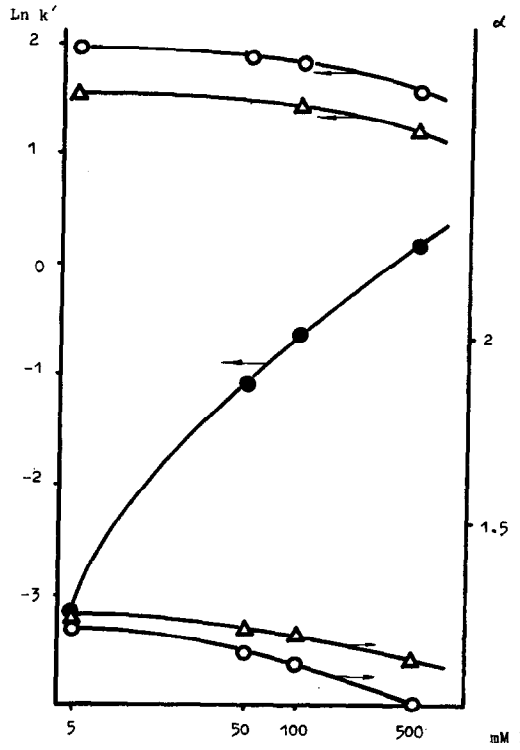

Fig. 3. Effect of orthophosphoric acid concentration on logarithm of capacity factors (ln k') of L–D isomers and the selectivity (α) of diastereoisomer separation. Column, Nucleosil 100 5-SA, 5 μm (200 × 4.0 mm I.D.); flow-rate, 0.40 ml/min. Compounds: ○ = L-Val–D-AlaP (**1**); △ = L-Ala–D-AlaP (**3**); ● = L-Leu–D-PheP(O-iso-C_3H_7)$_2$ (**5**).

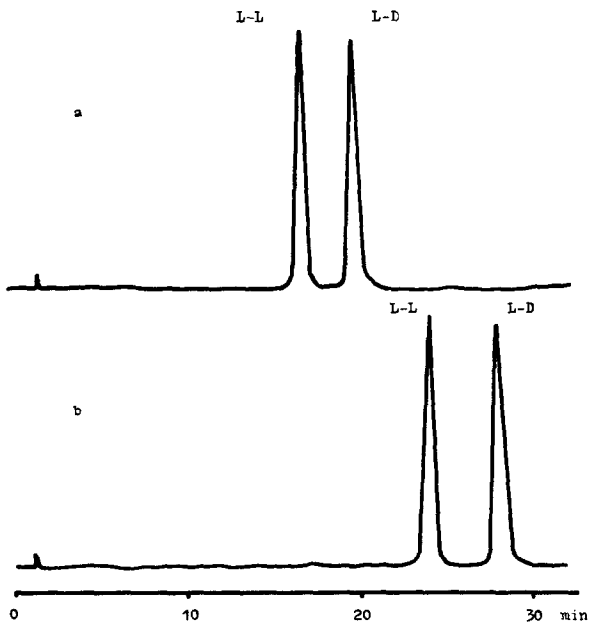

Fig. 4. Chromatograms of the separation of PP diastereomers. Column, Nucleosil 100 5-SA, 5 μm (200 × 4.0 mm I.D.); eluent, 0.1 mM H_3PO_4–methanol (95:5); flow-rate, 0.40 ml/min. (a) L-Ala–L,D-AlaP (**3**); (b) L-Val–L,D-AlaP (**1**).

sulphonate sorbent under conditions of cation-exchange chromatography is higher and the eluents with higher ionic strength should be used in this instance. A mobile phase containing 0.1 M potassium sulphate is the optimum for the separation of these PDs. Neither the retention nor the selectivity of the separation of these PDs is significantly influenced by the concentration of the phosphoric acid over the range 0.1–50 mM.

The selectivity and retention of the more hygrophilic PDs **1** and **3** decrease with increasing orthophosphoric acid content in the mobile phase (Fig. 3). Diastereoisomers of these PPepts were separated on column 2 by using an eluent containing 0.05–0.1 M orthophosphoric acid (Fig. 4).

Our attempts to separate diastereoisomers of the protected PPept **5** under conditions of cation-exchange chromatography on a sulphophenyl sorbent were unsuccesful. It can be assumed that the two hydrophobic isopropyl protecting groups of the PPept interact with aryl fragments of the sorbent. In this instance a fragment of the molecule with an asymmetric carbon atom is located in the mobile phase and it does not interact with the surface of the sorbent or contribute to the selectivity of separation. It is worth noting that the retention of protected peptides, in contrast to that of unprotected peptides, is considerably influenced by the concentration of phosphoric acid in the eluent (Fig. 3).

The results obtained permit optimum conditions and a sorbent for the complete separation of phosphonopeptide diastereoisomers to be chosen.

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