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Short communication

Enantiomeric separation of amino acids by capillary electrophoresis with α-cyclodextrin

Paweł Dżygiel[1], Piotr Wieczorek[1], Jan Åke Jönsson*

Department of Analytical Chemistry, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

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Abstract

A simple method for separation of underivatized aromatic amino acid enantiomers is presented. The influence of α-cyclodextrin concentration, applied voltage and background buffer pH on the separation were investigated to achieve the best conditions of separation. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Amino acids

1. Introduction

The purpose of this work was to devise a simple method of separating aromatic amino acid enantiomers without the need of labour intensive steps such as derivatization and addition of organic modifiers, in order to easily evaluate enantiomer-selective extraction procedures.

Separation of amino acids enantiomers by capillary zone electrophoresis (CZE) using cyclodextrins (CDs) has been reported in a number of articles, most of them devoted to separation of danslyated amino acids derivatives [1–7], although amino acids modified in other ways have also been studied [8–10].

Different types and sizes of CDs are used in CZE for chiral separation of various types of biologically active compounds, for example drugs, pesticides, hormones, etc.

CDs are cyclic oligosaccharides consisting of six, seven or eight (α-, β- or γ-CD) D-(+)-glucopyranose units in the shape of a truncated cone. This shape determines the presence of an inner cavity that, in turn, allows host–guest inclusion complexes. The guest molecule can be partially or fully incorporated in the hydrophobic interior of the CD. Typically, two enantiomers interacting with the CD have different binding affinities yielding an enantiomer separation. A practical reason for employing CDs in chiral separation is that their unique structure offers good selectivity along with sufficient solubility in the background electrolyte (BGE).

Optimization of an enantiomeric separation involves changing the type and/or concentration of CD, or adding other organic additives to the BGE.

Judging from the high interest shown in enantiomeric separation of amino acids, it is surprising that only a few works demonstrate the possibility of separating free amino acid enantiomers with direct

*Corresponding author.

[1]Permanent address: Institute of Chemistry, University of Opole, PL-45 052 Opole, Poland.

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detection. Fanali and Boček [11] have resolved tryptophan enantiomers with α-CD using a coated capillary in under 10 min. Similar results have been obtained by Altria et al. [12] but with different BGE composition and an uncoated capillary. In another work, Kuhn et al. [13] were also able to separate enantiomers of several amino acids using α-CD but only one amino acid at the time.

In the present work we have been trying to separate a mixture of free aromatic amino acids enantiomers by addition of α-CD to the BGE. The influence of applied voltage, pH and α-CD concentration on resolution of amino acid optical isomers was investigated. The obtained results are compared with respect to resolution and migration time.

2. Experimental

2.1. Chemicals

All D- and L-enantiomers of tryptophan, phenylalanine and tyrosine as well as α-CD were obtained from Sigma (St. Louis, MO, USA).

2.2. Apparatus, BGE and experimental procedure

Electrophoretic experiments were performed on P/ACE capillary electrophoresis system (Beckman, Palo Alto, CA, USA). CZE was performed in a fused-silica capillary 57 cm total length (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 (25+0.1°C). UV detection was used with a deuterium lamp operating at 214 nm (bandpass filter). Data analysis and collection were accomplished using a Compaq 386/20c computer and Beckman System P/ACE software version 2.0.

To obtain a required BGE solution, an appropriate amount of α-CD was dissolved in 0.1 *M* phosphoric buffer adjusted to desired pH with $HClO_4$. Experimental solutions were prepared by dissolving appropriate amounts of Trp, Tyr and Phe enantiomers together with 3 ml of 0.1 *M* hydrochloric acid in water in a 100 ml flask to give a solution of 1 m*M* amino acid hydrochlorides.

All water used was purified with a Milli-Q-RO4 system (Millipore, Bedford, MA, USA).

Experimental procedures were as follows: at the beginning and end of each working day, the capillary was washed with 0.1 *M* NaOH solution (15 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.

3. Results and discussion

Preliminary experiments indicated that background buffer adjusted to the required pH with perchloric acid instead of phosphoric acid results in a better separation, in line with the experiments performed by Fanali and Boček [11]. Hence in further experiments, the pH of background electrolyte was adjusted with perchloric acid.

3.1. Influence of α-CD concentration

As can be seen in Fig. 1, the separation of amino acid enantiomers strongly depends on the concentration of the chiral selector. An improvement in the quality of separation is evident with increasing α-CD concentration. It is possible to separate Trp and Phe enantiomers with 20 m*M* α-CD solution in BGE but the separation of D- and L-Tyr required higher (at least 40 m*M*) α-CD concentration. This might be due to the presence of the OH group attached to the benzene ring of the Tyr side chain, rendering Tyr less hydrophobic than Phe or Trp, thus causing a lower affinity of Tyr for the inner cavity of α-CD.

The alteration in α-CD concentration also has an impact on peak order in the electropherogram. The migration time of Tyr changes in relation to Trp and Phe. As the Tyr enantiomers are not as strongly bonded by α-CD as the enantiomers of Phe and Trp they exhibit a higher efficient electrophoretic mobility. As a consequence the migration times for both Tyr enantiomers are less affected by the α-CD concentration than the migration times of the other amino acid enantiomers.

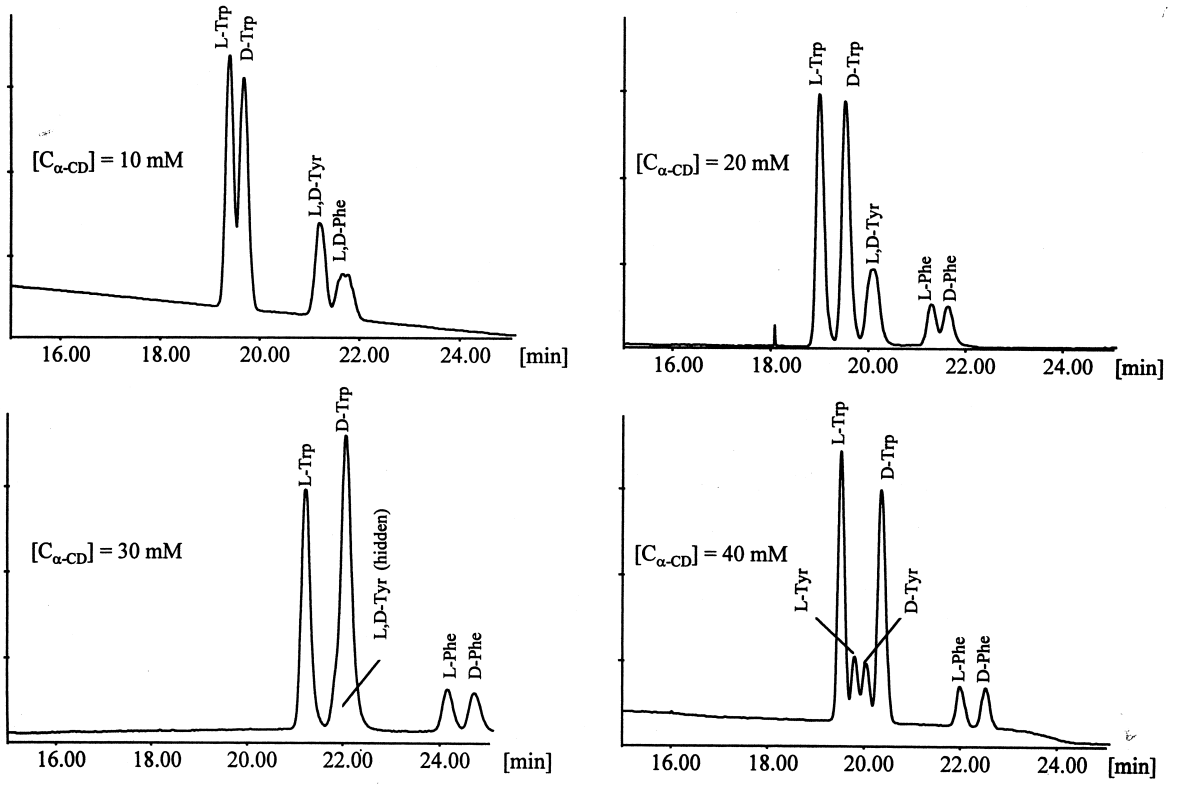

Fig. 1. Influence of α-CD concentration on separation. Conditions: background electrolyte pH, 2.5; applied voltage, 22.5 kV.

3.2. Influence of applied voltage

Fig. 2 shows the variation in migration times and peak widths with different electrical field strengths. As expected, higher voltage results in shorter migration times. It is possible to achieve a separation of each enantiomeric pair over almost the whole range of applied voltages (from 12.5 kV to 22.5 kV). However in the case of Tyr enantiomers, the electric field must be optimized to place both Tyr peaks between the L- and D-Trp peaks. This is possible because of the slight differences in the influence of applied voltage on the electrophoretic mobility of the investigated amino acids in the presence of α-CD.

3.3. Influence of background electrolyte pH

From Fig. 3, it can be seen that the separation of amino acid enantiomers is strongly dependent on pH of the background electrolyte, when other parameters are kept constant. Some difficulties emerge with the separation of Trp and Tyr peaks. Due to the shift in position of D- and L-Tyr peaks in relation to Trp enantiomers peaks, there is an overlap between L-Trp and L-Tyr at pH 2.5 and D-Tyr with D-Trp at pH 3.0. In comparison, the resolution of Tyr peaks at pH 2.75 is much better and only a slight overlap exists between D-Tyr and D-Trp. This is due to the relative change in efficient electrophoretic mobility between these two amino acids. However, the effects of pH and applied voltage are not independent. The same resolution (but with different migration times) was obtained at pH 2.5 with 22.5 kV (Fig. 1) and at pH 2.75 at 20 kV (Fig. 3).

3.4. Optimization of separation

Considering the results of the experiments described above, the best conditions were chosen in order to obtain the most efficient separation. The

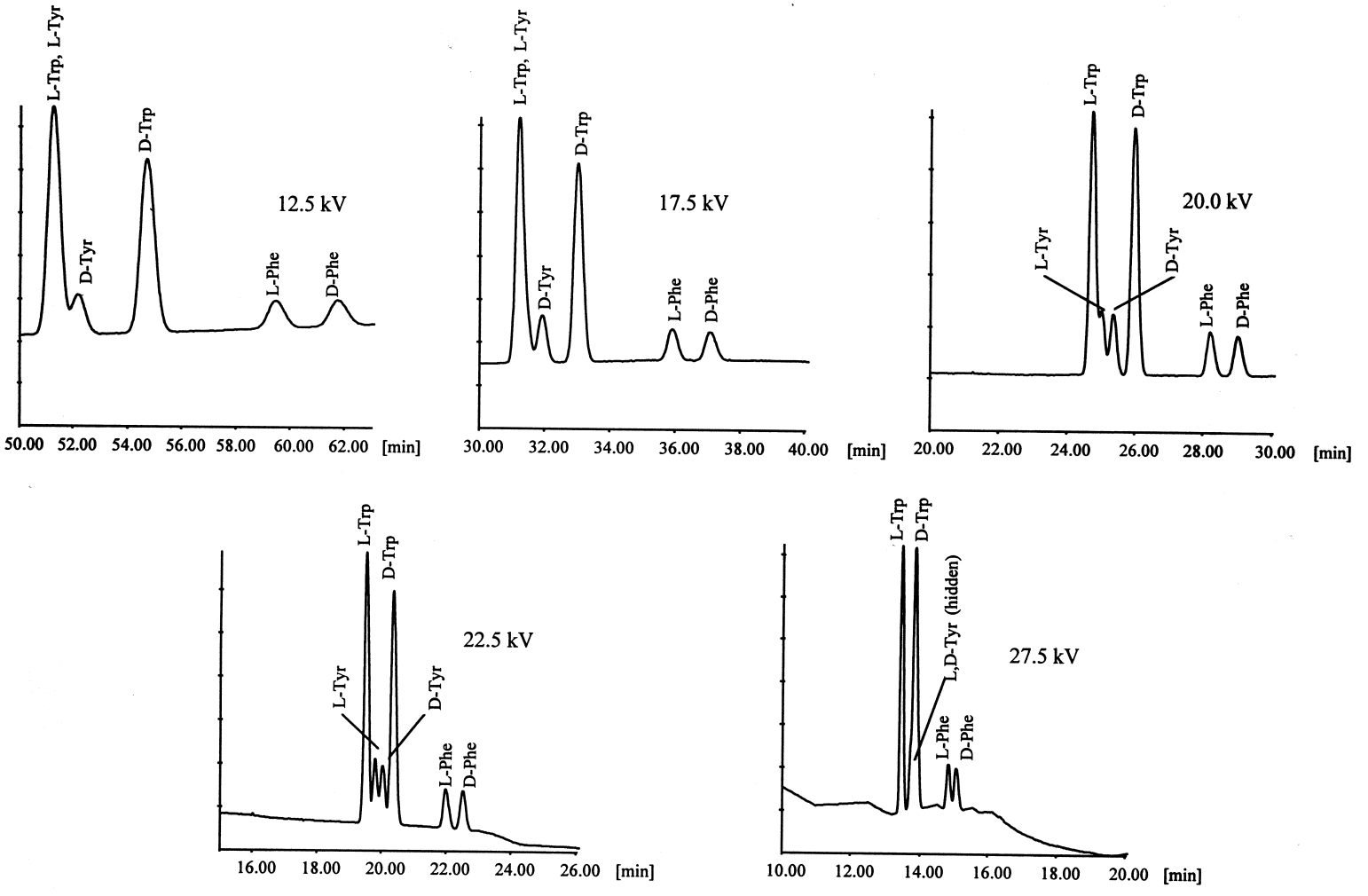

Fig. 2. Influence of applied voltage on separation. Conditions: background electrolyte pH, 2.5; [α-CD]=40 m*M*.

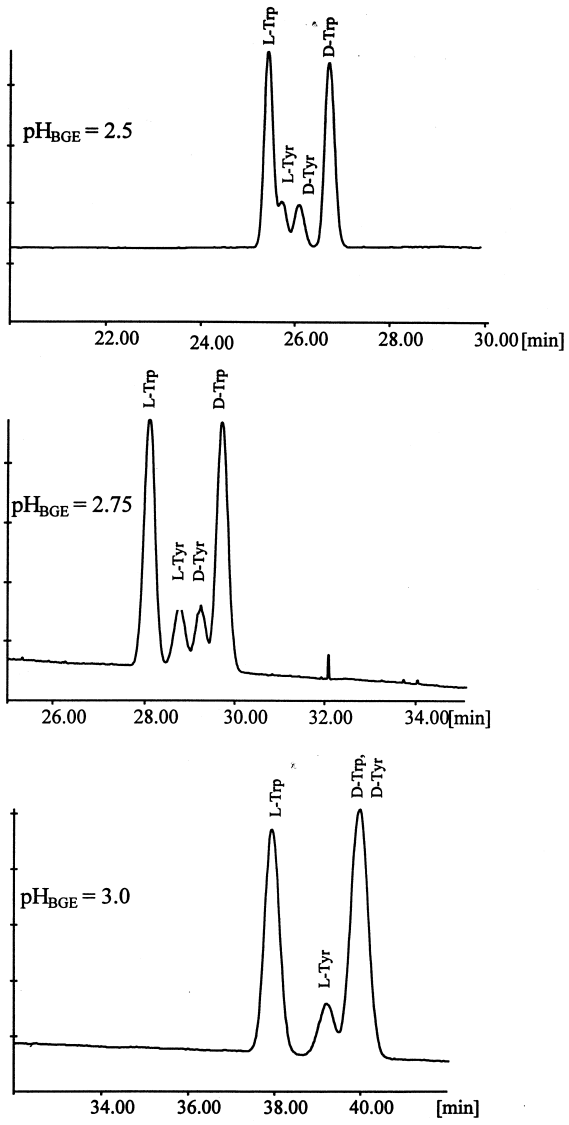

Fig. 3. Influence of background electrolyte pH on position of Tyr and Trp peaks. Conditions: applied voltage, 20 kV; [α-CD]=40 m*M*.

most suitable conditions are: applied voltage 22.5 kV, pH 2.5 and 40 m*M* α-CD concentration. An electropherogram obtained under these conditions is shown in Fig. 1 at 40 m*M*.

4. Conclusions

This work presents a potential method of separating a mixture of enantiomers of tyrosine, tryptophan and phenylalanine using simple conditions of analysis such as underivatized amino acids separated in phosphate buffer with α-CD addition and direct UV detection. By optimizing the applied voltage and pH of the background electrolyte, it is possible to separate overlapping peaks of tryptophan and tyrosine.

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References

[1] M. Tanaka, M. Yoshinaga, S. Asano, Y. Yamashoji, Y. Kawaguchi, Fresenius J. Anal. Chem. 343 (1992) 896.
[2] A. Werner, T. Nassauer, P. Kiechle, F. Erni, J. Chromatogr. A 666 (1994) 275.
[3] M. Yoshinaga, M. Tanaka, Anal. Chim. Acta 316 (1995) 121.
[4] M. Yoshinaga, M. Tanaka, J. Chromatogr. A 710 (1995) 331.
[5] I.E. Valkó, H. Sirén, M.-L. Riekkola, Chromatographia 43 (1996) 242.
[6] I.E. Valkó, H. Sirén, M.-L. Riekkola, J. Chromatogr. A 737 (1996) 263.
[7] G.N. Okafo, P. Camilleri, J. Microcol. Sep. 5 (1993) 149.
[8] D. Wistuba, H. Diebold, V. Schurig, J. Microcol. Sep. 4 (1992) 17.
[9] T. Ueda, R. Mitchell, F. Kitamura, T. Metcalf, T. Kuwana, A. Nakamoto, J. Chromatogr. 593 (1992) 265.
[10] W. Lindner, B. Böhs, V. Seidel, J. Chromatogr. A 697 (1995) 549.
[11] S. Fanali, P. Boček, Electrophoresis 11 (1990) 757.
[12] K.D. Altria, P. Harkin, M.G. Hindson, J. Chromatogr. B 686 (1996) 103.
[13] R. Kuhn, F. Stoecklin, F. Erni, Chromatographia 33 (1992) 32.