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Journal of Chromatography A, 1081 (2005) 9–18
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Analysis and separation of enkephalin and dalargin analogues and fragments by capillary zone electrophoresis

Veronika Šolínová[a], Václav Kašička[a,*], Tomislav Barth[a], Linda Hauzerová[a], Salvatore Fanali[b]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo 2, 16610 Prague 6, Czech Republic*
[b] *Institute of Chemical Methodologies, Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma I, Via Salaria Km 29,300, 00016 Monterotondo Scalo, Rome, Italy*

Available online 30 January 2005

Abstract

Capillary zone electrophoresis (CZE) has been applied to qualitative and quantitative analysis and separation of synthetic analogues and fragments of enkephalins ([Leu^5]enkephalin, H-Tyr-Gly-Gly-Phe-Leu-OH, [Met^5]enkephalin, H-Tyr-Gly-Gly-Phe-Met-OH), and dalargin (H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH), biologically active peptides with morphin-like effects acting as ligands for the opiate receptors in the brain. These oligopeptides (dipeptides to hexapeptides) were analyzed as cations in two acidic background electrolytes (BGEs), BGE I (100 mM H_3PO_4, 50 mM Tris, pH 2.25), BGE II (100 mM iminodiacetic acid, pH 2.30), and both as cations and anions in alkaline BGE IV (40 mM Tris, 40 mM Tricine, pH 8.10). Purity degrees of peptides, expressed in three different ways (relative peak height, relative peak area and relative corrected peak area), were determined by their CZE analyses in the above BGEs, and their values were compared with respect to the peak shapes and migration times of the main synthetic products and their admixtures. Selected analogues and fragments of enkephalins and dalargin were successfully separated by CZE in acidic isoelectric buffers, 100 and 200 mM iminodiacetic acid, pH 2.30 and 2.32, respectively. The effective electrophoretic mobilities at standard temperature 25 °C, and effective and specific charges of all analyzed peptides in the above three BGEs were determined. Correlation between effective electrophoretic mobility of the analyzed peptides and their charge and size (relative molecular mass) was investigated, which revealed different molecular shape of analyzed peptides in acidic and alkaline BGEs. In addition, the selected characteristics of the UV-absorption detector (noise, signal to noise ratio, sensitivity, and limits of detection and quantification) were determined.

Keywords: Capillary zone electrophoresis; Peptides; Enkephalins; Dalargin

1. Introduction

Capillary zone electrophoresis (CZE), one of the high-performance capillary electromigration methods, providing fast (few minutes) and high-efficient separation (10^5–10^6 theoretical plates per meter) of picomole to attomole amounts of analytes in the nanolitre sample volume, possesses a high application potential in the field of separations of peptides: it is broadly utilized for analysis, preparation and physicochemical and biochemical characterization of peptides both in the research and in practical applications in chemistry, biochemistry, biomedicine, biotechnology, pharmaceutical industry, food and feed industry and fishing farming, as documented in several recent reviews [1–7].

Enkephalins (ENKs), ([Leu^5]enkephalin [Leu^5]ENK), pentapeptide H-Tyr-Gly-Gly-Phe-Leu-OH, and [Met^5]enkephalin ([Met^5]ENK), pentapeptide H-Tyr-Gly-Gly-Phe-Met-OH, and dalargin (DLR), hexapeptide H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH, are biologically active peptides with morphin-like effects acting as ligands for the opiate receptors in the brain [8]. Enkephalins are fragments of nature opioid hormones, dynorphin A (17 amino acid residues) and dynorphin B (13 amino acid residues).

* Corresponding author. Tel.: +420 220 183 239; fax: +420 220 183 592.
E-mail address: kasicka@uochb.cas.cz (V. Kašička).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.046

Enkephalins and dynorphins originate from the two large precursor proteins, preproenkephalin and preprodynorphin [8]. They play significant roles in mediating stress and abatement of pain and are involved in temperature control, feeding behavior and respiration. These peptides and their analogues are used for the treatment of some mental illnesses (e.g. chronic schizophrenia, senile dementia of Alzheimer type).

CZE has been frequently used for analysis of enkephalins and dalargins, most often to check the purity of synthetic preparations of these peptides but also for their determination in biological fluids (serum, plasma, cerebrospinal fluids). Rational approach to selection and optimization of important experimental parameters (composition and pH of the background electrolyte (BGE), loading limit, capillary diameter and fraction collection) in analytical and preparative CZE of opioid peptides including enkephalin and dynorphin analogues with off-line mass spectrometric (MS) detection was reviewed by Lee and Desiderio [9]. [Leu^5]ENK, [Met^5]ENK and [desTyr^1-Leu^5]ENK were analyzed with high sensitivity (with limits of detection as low as 3–11 attomole) by CZE in citrate and phosphate BGEs using electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) [10]. Analogues of enkephalins and dalargin and other biopeptides, such as e.g. vasopressin, desmopressin, insulin-like growth factors, have been separated by CZE in strongly acidic BGE, 150 mM phosphoric acid, pH 1.8 [11]. These analogues were also analyzed and separated by capillary micellar electrokinetic chromatography (CMEKC) with micellar pseudophase formed by anionic detergent sodium dodecylsulfate (SDS) in alkaline BGEs (20 mM tetraborate, pH 9.2, and 20 mM phosphate, pH 8.8) or by cationic detergent cetyltrimethylammonium bromide (CTAB) in acidic BGE (50 mM phosphate, pH 4.1) [11–13]. Analogue of dalargin, [D-Tle^{2,5}]dalargin, was analyzed by CZE in 0.5 M acetic acid, pH 2.5, and then purified in preparative scale by free-flow zone electrophoresis [14]. Six analogues and fragments of enkephalins were separated and determined in biological matrices by multidimensional separation system consisting of on-line coupling of size-exclusion chromatography (SEC), reverse-phase C18 trapping column and CZE [15,16] and by liquid secondary ion mass spectrometry and tandem mass spectrometry (LSI-MS–MS–MS) [17]. Dynorphin peptide analogues were separated by CMEKC employing anionic (SDS), cationic (CTAB) and zwitterionic (CHAPS) surfactants [18]. Enkephalin-related peptides were derivatized by fluorescein isothiocyanate and then separated by CMEKC in borate BGE with SDS micelles and detected with laser-induced fluorescence detector [19]. [D-Pen^{2,5}]ENK (D-Pen is D-penicillamine or D-3-mercaptovaline) and [D-Ser^2,Thr^6]DLR were analyzed in rat serum by CZE in phosphate (pH 2.4) and borate (pH 8.3) buffers [20,21]. ENKs and their fragments were electrochemically detected as in-capillary formed copper complexes after their CZE separation [22]. ENK analogues were separated in tris-phosphate and sodium phosphate BGEs, pH 2.5, in capillary noncovalently coated with two layers of oppositely charged polymers [23]. [Leu^5]ENK, [Met^5]ENK and other peptide hormones were separated in three BGEs, pH 2.61, 2.85 and 10.0, and their pK_a were determined from the pH dependence of their electrophoretic mobilities in the broad pH range, 2–12, [24], and subsequently analyzed with CE-ESI-MS in 50 mM acetic acid and 50 mM formic acid, pH 2.85 [25].

The aim of this work was to perform qualitative and quantitative analysis of synthetic preparations of opioid peptide hormones, enkephalins and dalargin, and their analogs and fragments, by CZE both in acidic and alkaline BGEs. Suitable experimental conditions should be found for CZE separation of the mixtures of these structurally related peptides. In addition to the purity degree also some physicochemical characteristics of the analyzed peptides, such as effective electrophoretic mobilities, effective and specific charges should be determined, and the correlation between effective electrophoretic mobility of analyzed peptides and their charge and size (relative molecular mass) should be investigated.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. Iminodiacetic acid (IDAA) was obtained from Bachem (Bubendorf, Switzerland), Tris (tris(hydroxymethyl)aminomethane) was supplied by Serva (Heidelberg, Germany), phosphoric acid and dimethyl sulfoxide (DMSO) were obtained from Lachema (Brno, Czech Republic) and Tricine ([tris(hydroxymethyl)-methyl]-glycine) was from Merck (Darmstadt, Germany). Isophorone (3,5,5-trimethyl-2-cyclohexen-1-one) was supplied by Fluka (Buchs, Switzerland).

2.2. Peptides

The list of analyzed peptides and their abbreviations, sequences and relative molecular masses, M_r, are presented in Table 1. The oligopeptide fragments of ENKs, Tyr-Gly (YG) and Tyr-Gly-Gly (YGG), were obtained from Bachem (Bubendorf, Switzerland), the di- and tripeptide fragments of DLR, Tyr-Ala (YA) and Tyr-D-Ala-Gly (YaG), were purchased from Sigma (St. Louis, MO, USA). The analogs and derivatives of ENKs and DLR were prepared by solid phase synthesis [26]. The structure of DLR was altered by the substitution of leucine in position 5 by bulky amino acid such as D-tertiary leucine [27], by glycosylation [28] or iodination of tyrosine in position 1, by derivatization of the C-terminal arginine carboxyl group and by combination of these modifications.

2.3. Instrumentation

The capillary electrophoretic experiments were carried out in commercial P/ACE MDQ System (Beckman-Coulter,