Published in final edited form as:

Electrophoresis. 2016 September ; 37(17-18): 2297–2304. doi:10.1002/elps.201600006.

Separation of dynorphin peptides by capillary electrochromatography using a polydiallyldimethylammonium chloride gold nanoparticle-modified capillary

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Abstract

Dynorphin A (Dyn A) is an endogenous opioid peptide found in blood and CNS tissue at very low concentrations. Elevated levels of Dyn A due to different disease states, for example, neurodegenerative disease, have been linked to toxic nonopioid activity. Capillary electrophoresis (CE) is a powerful technique that can achieve high efficiency separations of charged analytes. However, CE has limited use for the analysis of basic proteins and peptides, due to their adsorption onto the inner surface of the fused silica at pHs below their pI. This adsorption can lead to a loss of efficiency, irreproducibility of migration times, and peak tailing. To obviate this problem, a polydiallyldimethylammonium chloride-stabilized gold nanoparticle-coated capillary was investigated for the separation of dynorphin metabolites. The positively charged gold nanoparticles (GNP) minimized unwanted adsorption of the positively charged peptides onto the surface of the fused silica capillary. Separation efficiency and resolution for opioid peptides Dyn A (1-6), Dyn A (1-7), Dyn A (1-8), Dyn A (1-11), and leu-enkephalin on the GNP-coated capillary column were evaluated under different experimental parameters. The best separation of Dyn A (1-17) and its fragments was achieved using a background electrolyte that consists of 40 mM sodium acetate buffer (pH 5) containing 5% GNP, a field strength of -306 V/cm, and a $75\text{ }\mu\text{m}$ i.d. capillary. The developed method was applied to the separation of tryptic peptide fragments of dynorphin A (1-17).

Keywords

Capillary electrochromatography; Dynorphin; Gold nanoparticles; Opioid peptides

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The authors have declared no conflict of interest.

1 Introduction

Dynorphin A (Dyn A) is an endogenous opioid peptide that is derived from the precursor peptide pre-prodynorphin [1, 2]. This peptide has high affinity for the κ -opioid receptor (KOR) [3]. Dyn A has been found to exhibit both antinociceptive and analgesic effects within the central nervous system [4] and is also involved in the body's immune response as well as cardiovascular and temperature regulation [5]. However, upregulation of Dyn A due to pathophysiological states (neurotrauma, neurodegeneration, or drug abuse) has been shown to cause nonopioid activity such as hyperalgesia, allodynia, and excitotoxicity [6]. These activities are mainly glutamatergic, and are mediated through the N-methyl-D-aspartate receptor (NMDA) [7-9]. Dyn A's toxic effects have been linked to neurotoxicity and cell death [7, 10].

Capillary electrophoresis (CE) can provide high separation efficiencies and short analysis times. It also requires low sample volumes and small amounts of run buffer [11]. However, one limitation for the analysis of proteins and peptides with high pI values is the adsorption of these species onto the inner-surface of fused silica capillaries, due to their interaction with ionized silanol groups [12]. This adsorption can lead to a loss of separation efficiency, irreproducibility of migration times, and peak tailing [13-15]. Coating of the inner surface can decrease the amount of adsorption of analytes onto fused silica capillaries [16]. The capillary coating can be either produced by covalent or physical bonding. Although the use of a permanent covalent coating does provide a more stable and reproducible electroosmotic flow (EOF), preparation of these capillaries can be tedious and time-consuming [16]. Physical coating using poly-charged polymers, for example polydiallyldimethylammonium chloride (pDDA/ PDADMAC), can produce a stable surface, although the bonding strength is weaker than that of covalent bonding [17]. These large poly-charged molecules offer several bonding sites to the capillary wall, ensuring a stable coating [16, 17]. Unfortunately, improving the separation through the modified surface is limited to a uniform (flat) surface and the chemical stability of the coating material [18]. Gold nanoparticles (GNP), due to their unique chemical and physical properties, are another material that has been used to coat capillaries for peptide and protein CE separations [19-22]. An advantage of the using nanoparticles in CE is that selectivity can be improved by controlling the size, shape, and type of coating used on the gold particle. All these parameters will influence the interaction of specific analytes with the GNP. The GNP also provide a greater surface area for these interactions compared to modified silica capillaries. Capillaries coated with citrate [23], didodecyldimethylammonium bromide [24], and octadecylamine [25] capped gold nanoparticles have been reported previously for capillary electrochromatography (CEC). Recently, Zhang *et al.* [26] reported a separation of heroin and basic impurities using gold nanoparticles that were stabilized using a cationic polymer of a quaternary ammonium salt polydiallyldimethylammonium chloride (pDDA-GNP).

The aim of this work was to evaluate GNP for the separation of the opioid peptide dynorphin A 1-17 and its metabolites (Table 1) using a CEC capillary coated with pDDA-GNP. It was shown that the pDDA-GNP were able to reduce the electrostatic undesirable adsorption of these positively charged peptides onto the surface of fused silica capillaries, leading to a highly efficient separation.

2 Materials and methods

2.1 Reagents and chemicals

Dynorphin A 1-17, dynorphin A 2-17, dynorphin A 1-13, dynorphin A 1-8 and dynorphin A 1-7 were purchased from Biomatik (Cambridge ON, Canada). Dynorphin A 1-6 and Dynorphin A 1-11 were purchased from Shanghai MoCell Biotech, Inc. (Shanghai, China). Leu-enkephalin was received from Alfa Aesar (Ward Hill, MA). Gold (III) chloride hydrate, 20% poly(diallyldimethylammonium chloride) [M.W. 200,000–350,000 Da] solution, sodium acetate trihydrate, sodium phosphate monobasic, sodium phosphate dibasic, and β -cyclodextrin were received from Sigma-Aldrich, (St. Louis, MO). Acetic acid, hydrochloric acid, methanol, and sodium hydroxide (molecular biology grade >98%) were purchased from Fisher Scientific (Fair Lawn, NJ). Sequencing grade modified trypsin was purchased from Promega (Madison, WI). All water used was Milli-Q grade (resistivity of 18 M Ω). Aqueous filter membranes (0.45 μ m) were purchased from Fisher Scientific. Polyimide-coated fused silica capillaries, 75 μ m i.d., 375 μ m o.d. were received from Polymicro Technologies (Phoenix, AZ).

2.2 Equipment

Analyses were performed on a Beckman Coulter P/ACE MDQ (Brea, CA) capillary electrophoresis system with a UV detector operating at 214 nm. Polyimide-coated fused silica capillaries, 75 μ m i.d., 375 μ m o.d. (Polymicro Technologies) were employed in the study. The total length of the capillaries (unless stated otherwise) was 49 cm, the effective length (from injection to detector) was 39 cm. A 0.5 cm detection window was made by burning off the outer polyimide capillary coating with Window Maker™ (Eatontown, NJ). Samples were introduced using hydrodynamic injection by applying 0.5 psi head pressure for 5 s. Electrophoretic separations were performed at applied voltage of (+/–) 10–20 kV. Following each run, the fused silica capillaries were rinsed with 0.1 M NaOH, water, and BGE for 3 min each. Modified capillaries were rinsed with BGE for 3 min before each sample injection. Data were recorded using the 32 Karat software (Beckman Coulter).

2.3 Synthesis of pDDA stabilized gold nanoparticles

PDDA-stabilized gold nanoparticles were synthesized based on the method described by Chen *et al.* [27]. A solution of 250 μ L of the polymer (4% pDDA solution), 200 μ L of 0.5 M NaOH, and 100 μ L AuHCl₄ (10 mg/mL) in 40 mL of MilliQ water was brought to boiling under vigorous stirring. The solution gradually turned from a colorless solution to a wine red color. When no more color change was observed, the heat was removed and the solution was left to stir at room temperature for an additional 15 min. The size and shape of the gold nanoparticles were confirmed using transmission electron microscopy (see supplement).

2.4 Preparation of buffer and standard solution

A stock solution of 100 mM sodium acetate buffer (pH 5) was prepared using sodium acetate trihydrate and acetic acid. The pH was adjusted using 1 M sodium hydroxide or 1 M hydrochloric acid. The BGE used for CE was achieved by further dilution of the stock solution and was filtered through a 0.45 μ m membrane filter before use. For the GNP-

modified capillary, 5% v/v pDDA-GNP was added to the BGE. A tryptic digest of Dyn A 1-17 was prepared as follows: trypsin was added to a solution of Dyn A 1-17 in 5 mM phosphate buffer (pH 7) at an enzyme:substrate ratio of 1:150 (mass/mass). The sample was then incubated at 37°C for up to 9 h. Small aliquots of the studied solution were collected hourly and then further diluted in the BGE.

2.5 Capillary column coating procedure

All solutions used for the modification of the capillary were freshly prepared. New fused silica capillaries were pre-conditioned by rinsing for 5 min each with 0.1 M HCl, water, methanol, water, 0.1 N NaOH, water, followed by a 10-min rinse with the BGE. The pDDA coating was done by rinsing the capillary with 0.1 N NaOH for 5 min followed by H₂O for 5 min; this was followed by rinsing the capillary 0.2% pDDA solution for 30 min and then rinsing the capillary with 0.02% pDDA in BGE for 10 min. A –12 kV potential was applied across the capillary for 10 min following the conditioning for equilibration of the BGE within the pDDA-coated capillary.

The pDDA-GNP coating procedure was as follows. The process was started by rinsing the capillary with 0.1 N NaOH for 20 min followed by H₂O for 15 min to ionize the silanol groups on the capillary surface. The capillary is then rinsed with (1:1) pDDA-GNP solution for 15 min, and the solution was held within the capillary for an additional 15 min (total 30 min). Next, the capillary was washed with H₂O to remove any excess unadsorbed GNP on the capillary surface. Finally, the modified capillary was rinsed with BGE for 15 min. A –12 kV potential was applied across the capillary for 10 min following the conditioning for equilibration of the buffer within the modified capillary (Fig. 1). Before each electrophoresis run, the modified capillary was rinsed with BGE for 3 min. The GNP-coated capillary was conditioned each day by rinsing with BGE for 10 min. Whenever changes of BGE components was required, the capillary was re-conditioned with the new BGE for 10 min. For overnight storage, the modified capillary was rinsed with MilliQ water for 10 min and then stored in water.

2.6 Electrophoresis procedure

Stock solutions of the peptides leu-enkephalin (Leu-ENK), dynorphin A (1-17), and the dynorphin A (1-17) fragments Dyn A (1-6), Dyn A (1-7), Dyn A (1-8), Dyn A (1-11), Dyn A (1-13), and Dyn A (2-17) were prepared at in MilliQ water at a concentration of 1 mg/mL and stored at –20°C in polypropylene microcentrifuge tubes. The peptides were then diluted to the required concentrations using the same BGE for each run. Samples were injected by applying 0.5 psi head pressure for 5 s. The separation voltage was between 12–20 kV in either normal or reverse polarity depending on capillary charge surface. To evaluate different electrophoresis conditions, five representation peptides—Leu-ENK, Dyn A (1-6), Dyn A (1-7), Dyn A (1-8), and Dyn A (1-11)—were chosen. These peptides mainly differ structurally in the number of cationic residues and size. Leu-ENK is neutral at pH5, and was used for the measurement of the EOF. In addition, a mixture of Dyn A (1-17) and seven other peptide fragments (Leu-ENK, Dyn A 1-6, Dyn A 1-7, Dyn A 1-8, Dyn A 1-11, Dyn A 1-13, Dyn A 2-17) was separated using pDDAGNP-coated capillaries.

3 Results and discussion

3.1 Separation of opioid peptides using unmodified fused silica

The separation of the mixture of the five opioid peptides was first attempted using an unmodified fused silica capillary and a BGE of 20 mM sodium acetate buffer (pH 5). The separation voltage was +12 kV using a capillary having a total length of 39 cm. Under these conditions, no peaks were observed in the electropherogram, most likely due to the irreversible adsorption of the peptides onto the surface of the fused silica capillary.

3.2 Separation of opioid peptides using a pDDA-coated capillary

The peptide mixture was then injected into a capillary coated with the cationic polymer (pDDA). This positively charged polymer adsorbed onto the negatively charged capillary wall, creating a positive surface. The excess positive charges from the polymer generated a significant EOF toward the anode ($\text{EOF} = -5.2 \times 10^{-4} \text{ cm}^2/\text{V.s}$). The surface also repelled the positively charged peptides and blocked the ionized silanol groups, thereby inhibiting adsorption of these peptides to the capillary wall.

Figure 2A shows the electropherogram obtained for the separation of five opioid peptides, Leu-ENK, Dyn A 1-6, Dyn A 1-7, Dyn A 1-8, and Dyn A 1-11. A BGE consisting of 20 mM sodium acetate buffer (pH 5) containing 0.02% v/v pDDA and a separation voltage of 15 kV (reverse polarity) was used. The separation of all five peptides was accomplished in less than 9 min with detection at the anode. The order of migration was Leu-ENK, Dyn A 1-6, Dyn A 1-8, Dyn A 1-7 and, finally, Dyn A 1-11. The resolution between each peak and its neighboring peak was calculated. For the peptides Leu-ENK, Dyn A 1-6, Dyn A 1-8, Dyn A 1-7, and Dyn A 1-11, R values were calculated to be 9.4, 6.93, 1.93, and 11.4 respectively. The migration order was predicted as follows: Leu-ENK is neutral at pH 5 and, therefore, migrated first. The remaining four dynorphin peptides all have one or more arginine groups in their chemical structures. Dynorphin 1-11 also has an additional lysine group. Although Dyn A 1-8 and Dyn A 1-7 have an equal number of arginine groups in their structures, the extra isoleucine group of Dyn A 1-8 led to an overall lower charge-to-size ratio and faster migration than for Dyn A 1-7.

3.3 Separation of opioid peptides using pDDA-gold nanoparticle-coated capillary

Figure 2B shows an electropherogram for the separation of the same five peptides using the pDDA-GNP-coated capillary. In this case, the BGE consisted of 20 mM sodium acetate buffer (pH 5) containing 5% pDDA-GNP. The gold nanoparticle-coated capillary exhibited a very fast anodal EOF separation ($\text{EOF} = -6.3 \times 10^{-4} \text{ cm}^2/\text{V.s}$), which is 20% greater than with a pDDA-coated capillary. We believe the pDDA-GNP adsorbed onto the capillary wall increased the surface area of the modified capillary surface, generating a larger net positive charge. The high EOF provided shorter analysis time and improved peak symmetry, with no change in the separation order from the previous method. The resolutions between each peak and its neighboring peak were Leu-ENK and Dyn A 1-6 ($R = 7.83$), Dyn A 1-6 and Dyn A 1-8 ($R = 5.31$), Dyn A 1-8 and Dyn A 1-7 ($R = 1.4$) and, finally, the resolution between Dyn A 1-7 and Dyn A 1-11 was calculated to be $R = 8.71$. The EOF measured for pDDA-gold nanoparticle-coated capillaries exhibited a notable same-day reproducibility (%RSD) of

0.76% and a between-day reproducibility of 1.68%. The pDDA-GNP capillaries were stable for several days, and the same capillary could be reused for up to 72 runs. We never had to discard a capillary due to instability.

3.4 Effect of BGE concentration

Different concentrations of sodium acetate—10, 20, 30, 40, and 50 mM (pH 5) containing a constant amount of GNP—were studied with the goal of improving resolution between each of the five peptides Leu-ENK, Dyn A1-6, Dyn A1-7 Dyn 1-8, and Dyn 1-11 (Figure 3). The increase in salt concentration lowers the double layer thickness and zeta potential, leading to slower EOF. Higher concentrations of sodium acetate in the BGE produced an improvement in the separation of all five peptides, but at the expense of longer run times.

3.5 Effect of capillary length

The effect of capillary length on the separation was evaluated in an attempt to improve the resolution of Dyn A 1-7 and Dyn 1-8. Using a constant field strength of 306 V/cm, two capillary lengths were evaluated—one with a total length of 39 cm and an effective length of 29 cm and a second with a total length of 49 cm and an effective length of 39 cm. The increase in capillary length led to an improvement in resolution, with an increase in the overall migration times of all five opioid peptides.

3.6 Effect of organic solvent and BGE additives

Methanol was then evaluated as an organic modifier in an attempt to improve the resolution of Dyn A 1-8 and Dyn A 1-7. Organic modifiers can reduce EOF due to their low dielectric constants [28]. Methanol was added to the BGE at a final concentration of 10% v/v. The effect of methanol on the separation is shown in Figure 4. The two peptides exhibited longer migration times, and there was some improvement in resolution ($R = 2.15$) compared to BGE without methanol ($R = 1.89$). In a previous report, Zhang et al. studied the influence of adding methanol as buffer additive. They found that concentrations less than 10% in the BGE caused instability of the separation current [26]. Higher concentrations of methanol were avoided in these studies since too much methanol may adversely affect the stability of pDDA nanoparticle capillary coating.

A structurally unmodified β -cyclodextrin (β -CD) was also investigated as a BGE modifier in an effort to improve resolution of Dyn A 1-8 and Dyn A 1-7. The concentration of β -CD in the BGE was 1.5 mM. Peptides with aromatic residues (tyrosine or tryptophan) are known to form inclusion complexes with cyclodextrin [29]. It was hoped that differences in the overall secondary structure of the two peptides could contribute to small differences in the affinities to the β -CD cavity and, thus, improve resolution [30-32]. Unfortunately, there was no substantial improvement in the resolution of Dyn A 1-8 and Dyn A 1-7 with or without β -CD in the BGE (40 mM sodium acetate containing 5% GNP) $R = 1.72$ and 1.7, respectively. However, an increase in resolution was seen with a BGE having an equal amount of β -CD at a higher sodium acetate concentration (BGE = 50 mM sodium acetate, $R = 1.78$) compared to the lower BGE concentration (20 mM sodium acetate, $R = 1.39$) containing equal concentrations of β -CD.

3.7 Separation of metabolites of dynorphin A 1-17

In vivo enzymatic degradation of Dyn A 1-17 produces fragments of the peptides with different pharmacological activity. An attempt to separate a standard mixture of Dyn A 1-17 and seven fragments of the opioid peptide (Dyn A 1-6, Dyn A 1-7, Dyn A 1-8, Dyn A 1-11, Dyn A 1-13, Dyn A 2-17 and leu-ENK) was studied at concentrations of 20, 40, 60, and 80 mM sodium acetate, each containing 5% v/v GNP (Fig. 5). The order of migration of the eight peptides was as follows: Leu-ENK, Dyn A 1-6, Dyn A 1-8, Dyn A 1-7, Dyn A 1-17, Dyn A 2-17, Dyn A 1-11, and Dyn A 1-13. The optimal BGE was determined to be 40 mM sodium acetate. The calculated resolutions were 9.72, 6.28, 1.69, 1.15, 1.71, 5.8, and 2.55. At lower concentrations of BGE, there was a decrease in resolution between Dyn A 1-8 and Dyn A 1-7 of 0.86 (peaks 3 and 4) and between Dyn A 1-17 and Dyn A 2-17 a decrease of 0.94 (peaks 5 and 6). Concentrations higher than 40 mM also showed good separations for both Dyn A 1-8 and Dyn A 1-7, but Dyn A 1-7 and Dyn A 1-17 (peaks 4 and 5) comigrated. The increase in concentration of the BGE decreased the double layer thickness, affecting the zeta potential and leading to slower EOF. This caused an increase in the migration time of all eight Dynorphin A fragments.

3.8 Separation of tryptic digest of dynorphin A 1-17

To demonstrate the separation, tryptic peptide fragments of Dynorphin A 1-17 were analyzed using the gold nanoparticle-coated capillaries. Figure 6 shows an electropherogram of the tryptic digest of Dyn A 1-17 at different time points. Peptide fragments were identified based on the migration times of the available standards of Dyn A 1-17 fragments. The electropherogram shows a decrease in the parent peptide Dyn A 1-17 (peak 4) and the appearance of its tryptic digest products Dyn A 1-7 (peak 3) and Dyn A 1-11 (peak 5), which are the N-terminus fragments of Dyn A 1-17. Two unknown peaks were also observed at approximately 3.8 and 5.2 min (asterisk). We believe the unknown peaks correspond to C-terminus fragments Dyn A 12-17 and Dyn A 8-17, respectively. This is based on data reported in the literature concerning the identification of a tryptic digest of dynorphin A 1-17 by LC-MS [33]. However, we were unable to confirm this conclusively due to the unavailability of the peptide standard. Using a gold nanoparticle-coated capillary, we were able to follow the progress of the digestion of dynorphin A 1-17 by trypsin over time. The gold nanoparticle-coated capillary was shown to be stable, and can be adapted for the analysis of complex biological samples.

4. Concluding remarks

Capillary electrophoresis separations of peptides having a high pI are challenging. This is due to the electrostatic adsorption of cationic peptides onto ionized silanol groups in the capillary wall. Here we have developed a method based on the use of pDDA stabilized gold nanoparticles as a static coating for the separation of dynorphin A 1-17 and its biologically active metabolites. The gold nanoparticle coating acts as a physical barrier between the analytes and the capillary wall. In addition, the charge-to-charge repulsion between the pDDA-stabilized gold nanoparticles and cationic groups of the peptide minimizes adsorption of the analyte to the capillary wall. Different background electrolyte parameters and additives were evaluated to improve the separation of dynorphin and its metabolites. Finally,

the approach was demonstrated for a tryptic digest of dynorphin A 1-17, which was analyzed using pDDA-stabilized gold nanoparticle-coated capillaries.

In the future, coupling of pDDA-stabilized gold nanoparticle-coated capillaries to more sensitive detection methods—for example, fluorescence or mass spectrometry—to achieve lower levels for the detection of pathophysiological levels of dynorphin A 1-17 and its metabolites will be undertaken.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge support for this work from the National Institutes of Health (grant R01 NS042929). A.M. Al-Hossaini was supported by King Saud University, Saudi Arabia. L. Suntornsuk was supported by Thailand-United States Educational Foundation (TUSEF/Fulbright Thailand). We would also like to thank Nancy Harmony for editorial assistance.

Abbreviation

Dyn A	Dynorphin A
Leu-ENK	Leu-enkephalin
GNP	gold nanoparticles
BGE	background electrolyte
pDDA/ PDADMAC	polydiallyldimethylammonium chloride
CEC	capillary electrochromatography

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Figure 1.
Illustration showing the interaction of capillary wall with A) a monolayer of polydiallyldimethylammonium chloride (pDDA); B) pDDA-stabilized gold nanoparticles.

Figure 2. Separation of (1) Leu-ENK, (2) Dyn A 1-6, (3) Dyn A 1-8, (4) Dyn A 1-7, (5) Dyn A 1-11. A) pDDA-modified capillary [BGE: 20 mM sodium acetate containing 0.02% pDDA (pH 5)]; B) pDDA-GNP-modified capillary [BGE: 20 mM sodium acetate containing 5% pDDAGNP (pH 5)]. HV: -15 kV, $L_T = 49$ cm, $L_D = 39$ cm, ID = 75 μ m. UV detection $\lambda = 214$ nm.

Figure 3.

The effect of BGE concentration [sodium acetate 10–50 mM, each containing 5% GNP (pH5)] on separation of Leu-ENK, Dyn A 1-6, Dyn A 1-8, Dyn A 1-7, and Dyn A 1-11. GNP-modified capillary; HV: -15 KV, $L_T = 49$ cm, $L_D = 39$ cm, ID= 75 μ m. UV detection $\lambda = 214$ nm.

Figure 4.

The effect of 10% (v/v) methanol on the resolution of (1) Dyn A 1-8, (2) Dyn A 1-7. GNP-modified capillary; HV: -15 kV, $L_T = 49$ cm, $L_D = 39$ cm, ID = 75 μ m. BGE: 50 mM sodium acetate containing 5% GNP and 10% CH_3OH (pH 5). UV detection $\lambda = 214$ nm.

Figure 5.

The effect of BGE concentration on separation of (1) Leu-ENK, (2) Dyn A 1-6, (3) Dyn A 1-8, (4) Dyn A 1-7, (5) Dyn A 1-17, (6) Dyn A 2-17, (7) Dyn A 1-11, and (8) Dyn A 1-13. (A) pDDA-GNP-modified capillary; HV = -15 kV, L_T = 49 cm, L_D = 39 cm, ID = 75 μ m; BGE: 20–80 mM sodium acetate containing 5% GNP (pH 5). (B) Electropherogram showing separation at BGE 40 mM sodium acetate containing 5% pDDA-GNP (pH 5). UV detection λ = 214 nm.

Figure 6.

Separation of peptides generated by a tryptic digest of dynorphin A 1-17. (A) 0 min, (B) 1 h, (C) 3 h, (D) 6 h, (E) 9 h. (1) Dyn A 12-17, (2) Dyn A 8-17, (3) Dyn A 1-7, (4) Dyn A 1-17, (5) Dyn A 1-11, based on migration time of standards. (*) Peak identity was predicted based on charge and size and migration time. GNP-modified capillary; HV = -15 kV, L_T = 49 cm, L_D = 39 cm, ID=75 μ m; BGE: 40 mM sodium acetate containing 5% GNP (pH 5). UV detection λ = 214 nm.

Table 1

Structure and pI of all eight opioid peptides used in this study.

Peptide	Structure	M.W. (g/mol)	pI
Dynorphin A 1-17	YGGFLRRIRPKLWDNQ	2147.52	11.41
Dynorphin A 2-17	GGFLRRIRPKLWDNQ	1984.34	12.13
Dynorphin A 1-13	YGGFLRRIRPKLK	1603.98	12.13
Dynorphin A 1-11	YGGFLRRIRPK	1362.65	12.12
Dynorphin A 1-8	YGGFLRRI	981.17	11.13
Dynorphin A 1-7	YGGFLRR	868.01	11.13
Dynorphin A 1-6	YGGFLR	711.82	9.84
Leu-enkephalin	YGGFL	555.63	5.93