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Evaluation of an on-capillary copper complexation methodology for the investigation of *in vitro* metabolism of dynorphin A 1–17

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Abstract

Dynorphin A 1–17 is an endogenous neuropeptide implicated in a variety of neurological disorders including Alzheimer's and Parkinson's diseases and neuropathic pain. Metabolites of this peptide can exhibit their own unique effects *in vivo*, and it is possible that one of these metabolites is responsible for the neurotoxicity. In this article, the use of CE for the separation of dynorphin A 1–17 from four of its metabolites is described. Buffer additives were investigated to eliminate peptide adsorption to the capillary wall and to improve resolution between closely related metabolites. On-capillary copper complexation was employed and was shown to improve separation efficiency as compared with the separation of native peptides. The method was then applied to *in vitro* dynorphin metabolism in human plasma as well as rat brain and rat spinal cord slices.

Keywords

Biuret reaction; CE; Copper; Dynorphin; Metabolism

1 Introduction

Dynorphin A 1–17 (Dyn A 1–17) is an endogenous opioid peptide with selectivity for the κ opioid receptor. The structures of Dyn and its major metabolites are shown in Fig. 1. The investigation of Dyn analogs as potential therapeutics for treatment of cocaine abuse and peripheral pain management has also revealed a neurotoxic component to this peptide's *in vivo* activity [1]. Elevated levels of Dyn have been implicated in a variety of neurodegenerative disorders including Alzheimer's disease [2], Parkinson's disease [3], and neuropathic pain [4], as well as stress and depression [5]. It is hypothesized that the neurotoxic effects are nonopioid in nature and may arise from activation of the *N*-methyl-D-aspartate receptor [6, 7]. Once released into the extracellular space, Dyn is subjected to enzymatic degradation with each metabolite exhibiting its own unique biological effects. It is possible that one of these metabolites is responsible for the neurotoxicity of Dyn. Therefore, methods to study the metabolism of Dyn *in vivo* are necessary to further elucidate the mechanisms of neurotoxicity.

The most common method for quantitation of Dyn *in vivo* is RIA. However, a major drawback to this approach is that cross-reactivity with metabolites can lead to falsely elevated results [8]. To circumvent this issue, chromatographic separation can be performed prior to the immunoassay. In one report, HPLC/RIA was employed to investigate the metabolism of Dyn A 1–13 in human blood [9]. This process took over 24 h and involved solid-phase extraction for sample cleanup and relatively long LC gradients (>20 min), followed by fraction collection prior to RIA. Another disadvantage of RIA is the use of expensive radioactive reagents and assay kits, as well as the costs associated with the disposal of radioactive waste. A variety of other methods have been used to quantify opioid peptides such as the enkephalins, endomorphins, and smaller Dyn peptides including, HPLC with UV detection [10], LC-MS [3, 11], CE-UV [12, 13], CE-LIF [13, 14], and CE-MS [15–19]. Qualitative and semi-quantitative MALDI methods have also demonstrated potential for monitoring Dyn peptides [20–23]. MS provides conclusive metabolite identification; however, the instrument requirements are expensive.

CE provides several advantages for monitoring peptides of biological and pharmacological interest [24, 25]. Highly efficient and fast separations are possible without the use of organic modifiers, separations can be performed at high pH, and various complexation strategies can be employed oncapillary. The small volume requirements of CE enable the analysis of volume-limited samples and allow the use of expensive separation additives. In this study, on-capillary copper complexation was investigated for the detection of Dyn peptides. Performing this complexation on-capillary is advantageous over pre- or postcolumn derivatization methods because sample dilution is avoided. This is especially important for the analysis of expensive compounds and volume-limited biological samples such as microdialysis samples.

In the late 1970s and early 1980s, Margerum and coworkers extensively characterized the coordination of Cu(II) with nitrogens in the peptide backbone [26–29]. This method is based on the biuret reaction, and can be used to improve UV detection of peptides as well as for the electrochemical detection of nontyrosine containing peptides. Weber's group exploited this method for postcolumn derivatization of peptides to enable electrochemical detection following LC separation [30–33]. In a separate report, Kennedy's group achieved picomolar detection limits of neuropeptides from microdialysis samples following precolumn derivatization with copper and both UV and electrochemical detection [34]. Previous study in our group has applied on-capillary copper complexation for the amperometric detection of enkephalin and angiotensin peptides [35–37]. A major advantage of this approach is that it is selective for peptides in the presence of amino acids because at minimum a tripeptide is required for complexation. Additionally, in contrast to many fluorescence derivatization techniques based on reactions with primary amines, *N*-terminally modified and cyclic peptides are also capable of forming complexes with copper.

In this article, a CE-based separation of Dyn A 1–17 and four previously identified metabolites are described. Phytic acid and various forms of CDs are investigated as run buffer additives to prevent peptide adsorption and improve resolution between metabolites. The copper complexation method is applied to *in vitro* metabolism in human plasma as well as rat brain and spinal cord. To the best of our knowledge, this is the first report of studying Dyn metabolism with CE.

2 Materials and methods

2.1 Reagents

All Dyn peptides (Dyn A 1–17, 2–17, 1–13, 1–8, and 1–6) were obtained from BaChem Biosciences (King of Prussia, PA, USA). Capillaries were obtained from Polymicro

Technologies (Phoenix, AZ, USA). Sodium tetraborate, phytic acid, D,L tartaric acid, copper(II) sulfate, β -CD, and (2-hydroxypropyl)- β -CD (HP- β -CD) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfobutyl ether- β -CD (SBE₄- β -CD) was obtained from Dr. Stella's laboratory at the University of Kansas. Mesityl oxide which was used as a neutral marker for EOF determination was also purchased from Sigma-Aldrich. Human plasma was obtained at Watkins Health Center (University of Kansas, Lawrence, KS, USA). Brain and spinal cord samples were obtained from male Wistar rats (Charles River, Wilmington, MA, USA).

2.2 Human plasma samples

Human plasma was obtained from a healthy volunteer at Watkins Health Center and used 1–2 h after withdrawal. Plasma was diluted 1:10 in 100 mM sodium tetraborate with 25 mM phytic acid, pH 9.0, to slow enzyme activity and prevent the capillary from clogging. Dyn A 1–17 was added at a concentration of 200 μ M and kept at room temperature to further retard enzyme activity. An initial aliquot was taken at $t=0$ and subsequent aliquots were taken approximately every 40 min, corresponding to a 25-min separation and flushes with NaOH and run buffer between runs. The run buffer for plasma analysis consisted of 100 mM sodium tetraborate, 25 mM phytic acid, 3 mM tartaric acid, 2 mM cupric sulfate, and 20 mM SBE₄- β -CD, pH 9.0.

2.3 Rat brain and spinal cord slices

The brains and spinal cords of male Wistar rats were removed on the day of analysis and kept in ice cold artificial cerebrospinal fluid prior to experiments. Animals that could no longer be used for studies due to their weight were graciously donated by Dr. Craig Lunte's laboratory at the University of Kansas. Tissue slices were prepared with a clean razor blade in a petri dish on a bed of ice. The tissue slices were exposed to Dyn at room temperature to slow enzyme activity. For brain samples, a 5 mm $\times$ 5 mm section was bathed in 100 mM sodium tetraborate with 25 mM phytic acid, pH 9.0, that was spiked with 200 μ M Dyn A 1–17. Immediately, an aliquot was removed and analyzed by the optimized copper complexation method. Subsequent aliquots were removed at approximately 40-min intervals to permit a 25-min CE separation followed by flushes with NaOH and run buffer. Similarly, a 5-mm length of spinal cord was prepared and bathed in buffer spiked with Dyn A 1–17. Aliquots were removed every 40 min. The run buffer for rat tissue slice analysis consisted of 100 mM sodium tetraborate, 25 mM phytic acid, 3 mM tartaric acid, 2 mM cupric sulfate, and 20 mM SBE₄- β -CD, pH 9.0.

2.4 CE analysis

Separations were performed on a Beckman Coulter PACE/MDQ (Brea, CA, USA) with UV detection at 200 and 280 nm. The system was controlled using 32-Karat software, and all subsequent data analyses were performed with this software as well. Fused silica capillaries (50 mm id $\times$ 360 mm od) were obtained from Polymicro Technologies. A 50 cm capillary (40 cm to detector) was used for native Dyn and a 60 cm capillary (50 cm to detector) was used for the Dyn–copper complexes. A small window was burned through the polyimide coating 10 cm from the capillary end for UV detection. Pressure injections were performed at 6.9 kPa for 5.0 s, and a separation voltage of 25 kV was applied with the anode at the injection end.

Buffers were prepared in 18M Ω deionized H₂O and adjusted to pH 9.0 with the addition of NaOH. Dyn stock solutions were prepared at a concentration of 1 mg/mL in 18M Ω deionized H₂O and kept frozen until use when they were diluted to desired concentrations in sodium tetraborate (50 or 100 mM) with 25 mM phytic acid (pH 9.0). Native Dyn separations were accomplished using an optimized background electrolyte consisting of 50

mM sodium tetraborate, 25 mM phytic acid, and 5 mM HP- β -CD, pH 9.0. Separation of the copper complexes was achieved using 100 mM sodium tetraborate, 25 mM phytic acid, 3 mM D,L-tartaric acid, 2 mM cupric sulfate, and 20 mM SBE₄- β -CD also pH 9.0.

3 Results

3.1 Separation optimization of native Dyn peptides

To study the metabolism of Dyn *in vitro*, this study focused on the development of an electrophoretic separation of Dyn A 1–17 from four previously identified metabolites – two of which, in particular, have been implicated in neurotoxicity and neuropathic pain (1–13 and 2–17). Initially, 50 mM sodium phosphate, pH 7.0, and 50 mM tetraborate, pH 9.0, were investigated as possible background electrolytes. Under these conditions, Dyn peptides were not detected. Upon repeated injections, very broad peaks with inconsistent migration times were observed; however, virtually no separation was seen between metabolites of interest. To reduce adsorption of the basic peptides onto the capillary wall, phytic acid was added to the background electrolyte. Phytic acid is a polyanion and acts as an ion-pairing agent to shield positively charged amino acid side chains from interaction with the silanol groups on the capillary wall [38–41]. This has been used previously by our group to prevent adsorption of substance P metabolites [42]. In addition to preventing peptide adsorption at the capillary wall, phytic acid has also been shown to improve resolution [39–41].

Ion pairing with highly basic peptides was also shown to result in longer migration times due to an overall change in their net charge [38–39]. Phytic acid concentrations of 15, 20, and 25 mM were evaluated in the run buffer and sample buffer, and 25 mM was determined to be sufficient to prevent analyte adsorption while keeping currents low enough to avoid excessive Joule heating. Although incomplete resolution of Dyn A 1–17 and 2–17 was still observed, higher phytic acid concentrations were not investigated due to high background currents resulting in increased noise and occurrence of bubble formation.

There are several aromatic residues present in Dyn that are capable of forming inclusion complexes with CD, including Tyr¹, Phe⁴, and Trp¹⁴. To further improve the resolution of Dyn A 1–17 and the des-Tyr metabolite, 5 mM HP- β -CD was included in the optimized run buffer. The final optimized separation is shown in Fig. 2. The UV response at 200 nm was linear over the concentration range of 2.5–50 μ M ($R^2 = 0.991$ –1.000, five concentrations, $n=3$ for each).

3.2 Separation optimization of copper-complexed Dyn peptides

It has been shown that, at basic pH, peptides can form complexes with copper(II) *via* nitrogens in the peptide backbone. Copper complexation has been shown to effectively improve UV sensitivity while also rendering peptides electroactive [26–37]. As shown in Fig. 3, complexation could be confirmed based on the shift in migration time seen with Dyn A 1–17 in copper-containing buffer (4.56 ± 0.14 *versus* 3.98 ± 0.08 min, $n=3$ for each). This later migration time is due to the increase in overall negative charge on the peptide following complexation due to resonance delocalization at the Dyn–copper coordinate sites [26–29]. Inclusion of copper in the run buffer causes minimal if any change in the EOF as shown by the consistent migration time of the neutral marker mesityl oxide (2.60 ± 0.03 *versus* 2.50 ± 0.03 min, $n=3$ for each).

The previously optimized 25 mM phytic acid was included in both run buffer and sample buffer during the optimization of the Dyn–copper complex separation. On-capillary complexation was performed using conditions previously optimized in our group employed for both enkephalin and angiotensin peptides [35–37]. The run buffer therefore included 50 mM sodium tetraborate, 25 mM phytic acid, 3 mM tartaric acid, and 2 mM cupric sulfate.

However, under these conditions, the resolution between all five peptides was decreased compared with native Dyn peptides. Copper complexation increases the overall negative charge on each peptide, reducing the differences in their mass-to-charge ratio, and thus, their electrophoretic mobilities. All of the Cu(II)-peptides migrated later, with Dyn A 1–6 and 1–8 co-migrating followed by 1–13, 1–17, and 2–17 co-migrating. This resolution was improved somewhat when the EOF was decreased by increasing the concentration of sodium tetraborate to 100 mM.

To further improve the separation of the Cu(II) complexed peptides, several different types of β -CDs were investigated. β -CDs consist of seven oligosaccharides in a cyclic arrangement. Inclusion complexes between hydro-phobic amino acid residues and the CD core form on capillary [43, 44]. In particular, three aromatic amino acid residues in the Dyn sequence – Tyr¹, Phe⁴, and Trp¹⁴ – could complex with CD. Changes in the substitution on the hydrophilic exterior of CDs alter their mobility on capillary. HP- β -CD was evaluated at both 5 and 10 mM, unsubstituted β -CD (β -CD) was investigated at 5 and 10 mM, and SBE₄- β -CD was evaluated at 2, 10, 15, and 20 mM. Not surprisingly, SBE₄- β -CD exhibited the most significant improvements in resolution due to its negative charge. Interaction with the hydrophobic core resulted in a higher negative electrophoretic mobility for the peptide complex. The effect of SBE₄- β -CD concentration on the separation is shown in Fig. 4. The optimal concentration of SBE₄- β -CD was determined to be 20 mM and was included in all subsequent studies. Under these conditions, UV response at 200 nm was linear over a range of 20–75 μ M ($R^2 = 0.9808 - 0.9915$, five concentrations, $n = 3$ for each). The optimized separation is shown in Fig. 5.

In general, copper complexation increased the peak heights and areas of Dyn peptides when detected at both 200 and 280 nm. Detection was most sensitive at 200 nm; however, 280 nm offered more selectivity for coppercomplexed peptides. The calculated peak efficiencies for native Dyn and Dyn–copper complexes are reported in Table 1. As can be seen in this table, the overall separation efficiency improved upon complexation.

3.3 Analysis of *in vitro* Dyn metabolism in biological tissues of interest

The analytical methods described above were then used to monitor the metabolism of Dyn A 1–17 *in vitro* in biological tissues of interest. Metabolic enzymes can vary greatly from tissue to tissue and species to species. The neurotoxic effects of Dyn peptides have generated an interest in the downstream effects of its metabolic processing since it may be a metabolite that is producing these effects. More specifically, the role of Dyn and its metabolites on the generation of neuropathic pain is of importance. In particular, the transport and metabolism of this peptide at the blood brain barrier is of interest and could provide insight into the mechanism of Dyn neurotoxicity.

In these studies, the metabolism of Dyn A 1–17 was investigated in several tissues, including human plasma, rat brain, and rat spinal cord, using the optimized copper complexation method. A 1:10 dilution of human plasma was spiked with Dyn A 1–17 (200 μ M). The metabolism was then monitored at 40-min intervals. As shown in Fig. 6, at time $t = 0$, only one peak is present in the electropherogram. The migration time of this peak corresponds with that of Dyn A 1–17. This peak was present only after spiking with Dyn and was not observed in the plasma blank. Therefore, it has been identified as the parent peptide. At time $t = 160$, two new peaks appear in the electro-pherogram. The early migrating metabolite is identified as Dyn A 1–13 and the later migrating peak as Dyn A 1–6. Again these peaks were identified based on injections of single metabolite standards and migration time comparisons.

A similar experiment was performed to examine the metabolism of Dyn in brain and spinal cord tissue slices. A 5 mm × 5 mm section of each tissue was removed and bathed in a buffer solution spiked with Dyn A 1–17 (200 μM). The metabolism of Dyn A 1–17 was monitored over time, and these electropherograms are shown in Figs. 7A and B. In the rat brain slice, at time $t = 0$, one dominant peak is observed for the parent peptide. The peptide is rapidly degraded in the presence of the brain tissue sample as can be seen by the complete absence of the 1–17 peak after 40 min. At this same time, a later migrating peak also appears, and based on migration time, it is identified as Dyn A 1–6. An early migrating peak also appears at this time point. The migration time of this new peak does not correspond to that of any of the metabolites that were investigated in this study. Interestingly, this early migrating unidentified metabolite also appears over time in the rat spinal cord sample. However, in the spinal cord slice, the metabolism of Dyn A 1–17 is considerably slower and only a small decrease in peak height of the parent compound is observed after 160 min.

4 Discussion

One of the limitations of CE for the analysis of basic peptides is the potential for analyte adsorption to the negatively charged silanol groups at the capillary wall [45]. Dyn is a highly basic peptide ($pI=11.4$) [46] that has been shown to adsorb rapidly to glass and plastic surfaces [47]. Although the use of polymer-modified capillaries has been explored for separations of basic drugs and proteins, these are expensive and time consuming to produce in house [48, 49]. A less expensive alternative employs an ion-pairing buffer additive. Phytic acid is a polyanion that acts to shield the positive charges on basic amino acid residues from the capillary wall. Kostel *et al.* were able to prevent substance P adsorption to capillaries by including phytic acid in the run buffer [42].

Resolution of closely related species can also be problematic in these applications. MEKC has been used to improve the resolution of opioid peptide analogs providing an additional mode of separation within a bare fused silica capillary [12]. In addition, CDs have been exploited as run buffer additives and have been shown to improve resolution for peptide metabolites and enantiomers of relevant pharmaceuticals [43, 44]. In this study, various forms of β -CD were investigated to improve resolution of closely related metabolites, and SBE $_4$ - β -CD was found to be optimal. The optimized separation has been used to examine *in vitro* metabolism of Dyn A 1–17 in human plasma as well as in rat brain and spinal cord.

Previous reports of Dyn metabolism have identified a variety of metabolites present in blood. Kreek's group investigated the *in vitro* metabolism of Dyn A 1–17 in both human and rhesus monkey blood using MALDI [20, 21, 23]. Human or monkey blood was spiked with Dyn A 1–17, and three major metabolites were identified, one opioid (Dyn A 1–6) and two nonopioid (Dyn A 2–17 and 7–17) peptides. These major metabolites were further degraded to 2–6, 8–17, 9–17, 11–17, 3–17, and 4–17. The Dyn A 1–6 and 7–17 fragments arise from cleavage at paired basic amino acid residues Arg(6)–Arg(7). The endopeptidase responsible for this metabolite has been termed Dyn A converting enzyme [50, 51]. Cleavage of the *N*-terminal tyrosine to produce Dyn A 2–17 is likely the result of nonspecific aminopeptidase activity [52, 53]. Although the metabolism products were similar in human and monkey blood, their time course differed considerably, with metabolism occurring much faster in rhesus monkeys.

The studies presented here detected the generation of two major metabolites over a 160 time period. The metabolites Dyn A 1–6 and Dyn A 1–13 were identified based on single injections of metabolite standards and correlating migration times. The presence of Dyn A 1–6 agrees well with the aforementioned studies performed in the Kreek lab [20, 21, 23], demonstrating the utility of CE in monitoring Dyn metabolism. It is thought that the

peptidase responsible for the cleavage of the *N*-terminal tyrosine is membrane-bound. The studies presented in this article were done in plasma as opposed to whole blood. This is a possible explanation for why Dyn A 2–17 was not observed in this study, but was reported previously [20, 21, 23].

Due to the neurotoxicity of Dyn A 1–17, its metabolism has also been studied in the brain. Several enzymes have been implicated in the metabolism of opioid peptides in the brain. In the early 1980s, an aminopeptidase from Sprague–Dawley rat brains was purified by Berg and Marks and shown to cleave the *N*-terminal tyrosine from enkephalin and Dyn peptides [52, 53]. However, the reactivity of this enzyme decreased with increasing size of the peptide, suggesting a conformational component to the availability of the tyrosine residue for enzymatic cleavage. As mentioned previously, the endopeptidase responsible for cleavage at the paired basic amino acids – Arg⁶ and Arg⁷ – is termed Dyn A converting enzyme and has been isolated and purified from human cerebrospinal fluid and human spinal cord tissue [50, 51].

Kreek's group investigated the metabolism of Dyn A 1–17 in the striatum of Fischer rats using microdialysis [22]. MALDI was employed for identification following direct infusion of Dyn A 1–17. Dyn A 1–7, 8–17, 1–6, and 9–17 were observed. Interestingly, the counterpart metabolites (7–17 and 1–8) were not detected. Andren's group has also investigated the metabolism of Dyn using microdialysis and nanoLC/ESI-TOFMS [3]. Their study compared Dyn metabolism in the brains of healthy Sprague–Dawley rats and those with unilateral 6-OHDA lesions to mimic Parkinson's disease. Several metabolites were found in the brains of the lesioned rats that were not present in healthy rats, exemplifying the importance of these metabolic events and their role in various neurological disorders.

To examine the metabolism of Dyn A in the central nervous system, *in vitro* studies with both rat brain and rat spinal cord slices were performed in this study. In the rat brain slice, the appearance of two metabolites was observed; however, only one could be identified by standard injections and migration time correlation. This metabolite is Dyn A 1–6 suggesting the presence of Dyn converting enzyme in the rat brain. The earlier migrating metabolite does not correspond to any of the metabolites investigated in this study. Interestingly, Dyn A 1–6 does not appear in studies with the rat spinal cord but, again, an unidentified early migrating metabolite is present. The time course of the metabolism in all three tissues also varies. Parent peptide is still present after 160 min in both the plasma and the spinal cord. In the rat brain, however, the peak for Dyn A 1–17 is completely absent by the 40-min time point. Differences in the metabolic kinetics between species and tissues have been previously reported. For example, Kreek's group found the metabolism of Dyn A 1–17 to be much faster in monkey blood than in human blood [20, 23].

5 Concluding remarks

A method for on-capillary copper complexation of Dyn peptides has been developed. The method described here demonstrates the usefulness of CE for examining peptide degradation in a single assay where the parent peptide and metabolites are detected simultaneously following electrophoretic separation. It is clear that the metabolism of Dyn is a complex process that varies between tissues and species. CE provides a convenient format for the separation and detection of the parent peptide and its metabolites.

The use of on-capillary complexation improves the efficiency of these separations and has the potential to render even the des-tyrosine metabolites electroactive, making amperometric detection feasible. Future study will focus on electrochemical detection of the peptides to improve limits of detection. Additionally, transferring this separation to a microchip device for even faster analysis is being explored. In these studies, metabolism was slowed by

diluting the plasma and performing the analysis at room temperature. In a microchip format, these steps could potentially be avoided, enabling a more physiologically relevant investigation of Dyn metabolism. The length of time for the CE runs is the limiting factor with respect to temporal resolution, and microchip electrophoresis would improve this from 40 min to less than 5 min. This miniaturized format would enable direct coupling to microdialysis sampling, making near real-time monitoring of Dyn metabolism *in vivo* possible.

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Abbreviations

Dyn A 1–17	dynorphin A 1–17
HP-β-CD	(2-hydroxypropyl)- β -CD
SBE₄-β-CD	sulfobutyl ether- β -CD

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Figure 2.

Optimized separation of native Dyn peptides. Buffer: 50 mM sodium tetraborate, 25 mM phytic acid, 5 mM HP- β -CD, pH 9.0. Peptides are 100 μ M in 50 mM sodium tetra-borate, 25 mM phytic acid, pH 9.0.

Figure 3.

Comparison of migration time of Dyn A 1-17 with (-----) and without (_____) copper run buffer. Native run buffer: 50 mM sodium tetraborate, 25 mM phytic acid, pH 9.0, copper run buffer: 50 mM sodium tetra-borate, 25 mM phytic acid, 2 mM CuSO_4 , 3 mM tartaric acid, pH 9.0. Peptides were 100 μM .

Figure 4.

Effect of SBE-β-CD on relative migration time. Run buffer contained 100 mM sodium tetraborate, 25 mM phytic acid, 3 mM tartaric acid, 2 mM cupric sulfate, and specified amount of SBE-β-CD. Peptides are 100 μM in 100 mM sodium tetraborate, 25 mM phytic acid. Migration times are based on the averages of triplicate runs, relative to mesityl oxide a neutral marker.

Figure 5.

Optimized separation of Dyn-copper complexes. Buffer: 100 mM sodium tetraborate, 25 mM phytic acid, 20 mM SBE- β -CD, pH 9.0. Peptides are 100 μ M in 100 mM sodium tetraborate, 25 mM phytic acid, pH 9.0.

Figure 7. Metabolism of Dyn A 1-17 in rat brain (A) and rat spinal cord (B). Metabolites were identified based on the standard single injections of each peptide. X, Unidentified metabolite.

Table 1Effect of copper complexation on separation efficiency^{a)}

Peptide	Native Dyn (<i>N</i>)	Dyn-copper complexes (<i>N</i>)
Dyn A 1–6	1158	18 828
Dyn A 1–8	17 781	16 808
Dyn A 1–13	4124	4736
Dyn A 1–17	4960	9707
Dyn A 2–17	2252	8968

^{a)} Each peptide was 100 μ M ($n=3$) in sodium tetraborate buffer with 25 mM phytic acid. Run buffer conditions: Native (50 mM sodium tetraborate, 25 mM phytic acid, 5 mM HP- β -CD, pH 9.0, Complexes (100 mM sodium tetraborate, 25 mM phytic acid, 2 mM CuSO₄, 2 mM tartaric acid, 20 mM SBE4- β -CD, pH 9.0). Efficiency (*N*) was calculated using the following equation: $N=16(R/w_b)^2$.